

The American Journal of Medicine

VOL. XVIII

MARCH, 1955

No. 3

Editorial

The Pathogenesis of Fever

"FEVER occurs in many different types of disease, including such diverse entities as infections, neoplastic diseases, hemolytic crises, vascular accidents, and mechanical injuries. The way in which these various processes affect temperature regulation is unknown, but the suggestion has been made that some agent, liberated from injured cells, acts on the thermoregulatory centers and disturbs their function."

This clear statement of one of medicine's most fascinating unsolved problems appears in a brief abstract published in the *Journal of Clinical Investigation* in 1948.¹ Its author reported experiments in which he had attempted to extract pyrogenic substances from a variety of rabbit cells, including erythrocytes, lymphocytes, monocytes and polymorphonuclear leukocytes. Only from the last named was he able to obtain an actively pyrogenic extract. "It seems possible," he concluded, "that the liberation of material such as that present in the polymorphonuclear leukocyte of the rabbit plays a role in the pathogenesis of fever in certain diseases in man."

These important studies of Beeson marked the beginnings of an intensive effort on the part of several groups of investigators to gain further insight into the causes of fever. To be sure, Menkin had described "pyrexin" in 1945—a relatively heat stable factor extracted from experimental exudates and capable of producing fever when injected intravenously into rabbits.² But, as recently emphasized by Bennett and Beeson, the validity of Menkin's observations is open to question because he failed to employ the technics necessary to exclude the presence of extraneous pyrogens.³

¹ BEESON, P. B. *J. Clin. Investigation*, 27: 524, 1948 (abstract).

² MENKIN, V. *Arch. Path.*, 39: 28, 1945.

³ BENNETT, I. L., JR. and BEESON, P. B. *J. Exper. Med.*, 98: 477 and 493, 1953.

It is well known that body temperature is controlled primarily by thermoregulating centers in the brain.⁴ But what are the sources and nature of the stimuli which act upon the thermoregulatory centers to cause the fevers of disease? This basic question remains unanswered. Only in the case of neurogenic fever, resulting from direct injury to the central nervous system, is the origin of the febrile response clearly understood.

Most modern experiments relating to the pathogenesis of fever have been carried out with rabbits injected intravenously with pyrogenic substances of bacterial origin.⁵ Such injections cause a characteristic rise in body temperature, but only after an appreciable latent period. This observation has led to the theory that a secondarily released endogenous substance, rather than the originally injected pyrogen, acts upon the thermoregulatory centers to initiate fever. Evidence has also been obtained that the injected pyrogen combines with a factor in normal serum before exerting its pyrogenic effect.⁶ Preincubation of pyrogen with normal rabbit serum shortens the latency of the fever response. In addition to this "accelerator" factor, serum "inhibitors" have been demonstrated in the blood of rabbits given repeated daily injections of pyrogen.⁷ Such rabbits become "tolerant" to the pyrogen and exhibit a greatly diminished febrile response. Because it can be reversed by reticulo-endothelial blockade, pyrogen tolerance has also been attributed to rapid removal of the injected pyrogen from the circulation by

⁴ GRANT, R. *Ann. Rev. Physiol.*, 13: 75, 1951.

⁵ BENNETT, I. L., JR. and BEESON, P. B. *Medicine*, 29: 365, 1950.

⁶ GRANT, R. and WHALEN, W. J. *Am. J. Physiol.*, 173: 47, 1953.

⁷ FARR, R. S., CLARK, S. L., JR., PROFFITT, J. E. and CAMPBELL, D. H. *Am. J. Physiol.*, 177: 269, 1954.

phagocytic cells of the animals' reticuloendothelial system.⁸

The question may rightfully be raised as to whether such experimentally induced fever has more than a remote relationship to the fevers encountered in clinical medicine. Admittedly, pyrogen fever serves as a convenient laboratory model. But in addition, since the injected pyrogen apparently does not itself cause the febrile reaction, the possibility exists that the temperature controlling centers of the brain are stimulated through the same basic mechanism as that which operates in the fevers of infections, neoplasms and other diseases. The nature of the common mechanism, if indeed there is one, remains unknown. However, the suggestion that initiation of fever involves the release by injured cells (including leukocytes) of an endogenous pyrogen, which in turn acts upon the central nervous system, is not without logic. The recent confirmation of Beeson's original finding of an extractable pyrogenic factor in polymorphonuclear leukocytes adds support to the hypothesis.³

To the experimental evidence favoring the "cell-injury theory" the following pertinent observations have lately been added:

First, the well known leukopenic response which regularly precedes fever induced by typhoid vaccine has been shown to occur at a relatively constant time interval in advance of the initial rise in temperature.⁹ When latency between the injection of the vaccine and the onset of fever is short, the leukopenia occurs early; conversely, when the latent period is prolonged, the leukopenic reaction is delayed. Variations in the length of the latent period are readily produced by taking advantage of the serum "accelerator" and "inhibitor" phenomena already referred to. Also, contrary to the findings of others,^{8,10} the observation has been made that the leukopenic response to typhoid pyrogen is negligible in rabbits previously made tolerant to the vaccine.⁹

Secondly, leukocytes obtained from the circulation of rabbits previously injected with pyrogen have been shown to behave abnormally when studied *in vitro*.¹¹ Their failure to migrate from the buffy coat of the blood in tissue culture and in slide cell preparations suggests cell injury.

⁸ BEESON, P. B. *J. Exper. Med.*, 86: 29 and 39, 1947.

⁹ ATKINS, E., ALLISON, F., JR. and WOOD, W. B., JR. To be published.

¹⁰ CLUFF, L. E. *J. Exper. Med.*, 98: 349, 1953.

¹¹ BERTHRONG, M. and CLUFF, L. E. *J. Exper. Med.*, 98: 331, 1953.

The abnormality is demonstrable within five minutes of the time the pyrogen is injected and lasts for several hours. It fails to occur in the presence of pyrogen tolerance.¹⁰

Thirdly, a circulating fever-producing substance, which may prove to be of endogenous origin, has been recently detected in the blood of "sensitized" but non-tolerant rabbits two hours after the injection of pyrogen.¹² The existence of this as yet undefined "two-hour factor" has been demonstrated by passive transfer experiments in which the blood of pyrogen-injected donor rabbits is tested for fever-promoting properties in normal recipients. Whereas the blood of virgin donors is actively pyrogenic for at least two hours after the injection of exogenous pyrogen, that of sensitized donors has lost all pyrogenicity within thirty minutes after the injection but has regained its ability to produce fever by the end of two hours. The temporary disappearance of pyrogenicity from the blood of such sensitized donors, together with the fact that the blood of tolerant rabbits at no time exhibits demonstrable quantities of transferable pyrogen, suggests that the two-hour factor may be quite distinct from the artificial pyrogen originally injected. If on further study the two-hour factor proves to be endogenous, its source may well be found to reside in the donor animals' leukocytes and other cells which have been damaged by the foreign pyrogen.

At least two additional experimental observations, on the other hand, have been cited as evidence against the cell-injury theory. The first concerns the failure of severe agranulocytosis to lessen the fever responsiveness of rabbits to bacterial pyrogens. This fact, recorded by Bennett and Cluff,¹³ was later supplemented by the finding that exudates produced in agranulocytic rabbits and therefore containing relatively few polymorphonuclear leukocytes are nonetheless actively pyrogenic.³ The second observation deals with the failure of antipyretically effective doses of cortisone to influence the leukopenic response of rabbits to intravenous pyrogen.^{14,15} This dissociation of fever from leukopenia has led Bennett and Beeson to conclude that, "There is no evidence that the leukocyte changes after

¹² ATKINS, E. and WOOD, W. B., JR. To be published.

¹³ BENNETT, I. L., JR. and CLUFF, L. E. *Proc. Soc. Exper. Biol. & Med.*, 81: 304, 1952.

¹⁴ BENNETT, I. L., JR. and BEESON, P. B. *Bull. Johns Hopkins Hosp.*, 93: 290, 1953.

¹⁵ ATKINS, E., ALLISON, F., JR., SMITH, M. R. and WOOD, W. B., JR. *J. Exper. Med.*, in press.

pyrogen administration are related to the production of fever."¹⁴

Such a conclusion, however, is not necessarily supported by either of these observations. For the first may be interpreted as merely indicating that injured cells other than granulocytes may produce endogenous pyrogen,^{3,12} and the second may be due to a direct effect of cortisone upon the thermoregulatory centers of the brain.¹⁵

Manifestly the cell injury theory is only an

attractive working hypothesis based upon indirect evidence. It would seem possible that more direct information regarding its validity may come from further study of the two-hour factor. If this substance is ultimately demonstrated to have the same properties as those of the pyrogenic substance extractable from leukocytes, a crucial link will have been added to a growing chain of evidence.

W. BARRY WOOD, JR., M.D.

Clinical Studies

Studies of Hepatic Function in Diabetes Mellitus, Portal Cirrhosis and Other Liver Diseases*

A Correlation of Clinical, Biochemical and Liver Needle Biopsy Findings

I. Diabetes Mellitus

A. BOGOCH, M.D., W. G. B. CASSELMAN, M.D., † A. KAPLAN, M.D. and H. L. BOCKUS, M.D.
Vancouver, B. C. *Toronto, Canada*

Philadelphia, Pennsylvania

THE most evident biochemical manifestations of diabetes mellitus reflect disturbed carbohydrate metabolism. Coincidentally or subsequently, alterations in fat, protein and electrolyte metabolism occur.¹ Although the role of the liver in the mechanisms of these disorders has been studied extensively, our knowledge is still meager owing to the lack of precise methods of assessing many of the diverse activities of this organ. The reported incidence of hepatic dysfunction in diabetes has ranged from low values^{2,3} to as high as 38.9 per cent.⁴ Such apparent differences may be related to variation in the interpretation of liver function tests, the selection of patients, or the fallibility of statistically unsupported impressions. In adults with untreated, complicated diabetes⁵ and in poorly controlled juvenile diabetics⁶ hepatomegaly may be demonstrable. Usually this is associated with fatty metamorphosis which in some instances may be striking without clinically apparent enlargement of the liver. Thus there is not only biochemical but also clinical and morphologic evidence suggesting hepatic dysfunction. Most observers believe that this is either incidental to or an aftermath of the diabetic state.

The investigations reported here provide further information regarding the liver in diabetic patients. The results of clinical and biochemical investigations of sixteen diabetic patients have been compared with histologic and histochemical observations on hepatic tissue aspirated by needle biopsy. To permit correlation of corresponding constituents, the samples of blood and liver were obtained simultaneously. For comparison, similar studies were made of seventeen non-diabetic patients with other liver diseases.

MATERIAL AND METHODS

The thirty-three patients in this study were first thoroughly evaluated clinically. The relevant clinical data are presented in Table I for the diabetics and in Table VI for the non-diabetics with portal cirrhosis. Any evident factors possibly affecting hepatic function at the time of biopsy are listed.

Laboratory Studies. A blood sample from the left ante-cubital vein and a liver biopsy were taken at the same time. The results of laboratory studies of the blood samples are recorded in Tables II and VII. The battery of tests was repeated serially throughout the investigation of

* From the Departments of Medicine and Gastroenterology, Graduate Hospital, University of Pennsylvania, and the Banting and Best Department of Medical Research, University of Toronto.

† Senior Medical Research Fellow, National Research Council, Canada.

each patient. Usually, the biochemical findings at other times during their hospitalization were similar to those recorded except when an apparent reason for difference existed. The following determinations were made in the laboratories of the Graduate Hospital: total and direct-reacting serum bilirubin,⁷ blood glucose,⁸ serum alkaline phosphatase,⁹ cephalin-cholesterol flocculation test,^{10,11} thymol turbidity test,¹² thymol flocculation test,¹³ serum colloidal gold reaction¹⁴ (Boerner reagent¹⁵), scarlet red test,¹⁶ serum albumin and globulins and total serum proteins,¹⁷ serum amylase¹⁸ and lipase.¹⁹ In most instances duplicate determinations of serum fatty acids,²⁰ phospholipids²¹ and total and free serum cholesterol²² were made in the Research Laboratory, Jefferson Medical College, Philadelphia, under the direction of Dr. I. J. Pincus. The cholesterol ester fractions noted in Tables II and VII were calculated by subtracting the free from the total cholesterol value. The remaining serum lipid determinations were made in the Graduate Hospital and are given within parentheses. These include total serum lipids²³ and total serum cholesterol and the esterified fraction.²⁴ The free cholesterol was calculated as the difference between the total and ester values.

In some cases the serum proteins were studied qualitatively by paper electrophoresis²⁵ and, occasionally, by electrophoresis using the Tiselius method.

Unless otherwise noted, bromsulfalein retention²⁶ (5 mg./kg. at forty-five minutes) was estimated within three days before or after the liver biopsy.

Liver Biopsies. A liver biopsy was not taken unless the prothrombin time was normal. Where necessary, parenteral vitamin K was administered. In addition, it was necessary to give two patients (No. 4 and 5) transfusions of 500 ml. of blood on the day before their biopsies were taken. The biopsies were obtained with a Franseen needle using the intercostal approach. From patient No. 5 the specimen of liver tissue was obtained at peritoneoscopy. The tissue was aspirated into a syringe containing cobalt-formal-calcium²⁷ fixative.

For reasons other than the liver biopsy, patient No. 6 received 500 ml. of blood on the day before biopsy as well as 1,000 ml. during the preceding two weeks. A total of 1,750 ml. of blood was given over four days to patient No. 15 two weeks before his biopsy was taken. These details are recorded in view of their possible

effects upon the biochemical and histochemical observations.

Histologic and Histochemical Studies. The liver tissue obtained at biopsy was divided into three portions. Two were fixed in 80 per cent ethanol for paraffin sections and one in cobalt-formal-calcium for frozen sections. The latter and one of the other two portions were sent by air mail to Toronto.

For histologic studies in Toronto, separate paraffin sections were stained with hematoxylin and eosin, a modification of the Mallory stain for connective tissues and a silver method for reticulum. In Philadelphia paraffin sections of the second portion fixed in ethanol were stained with hematoxylin and eosin. Later all histologic findings were compared to insure that all abnormalities were recognized. The results are presented in Tables III and VIII.

The histochemical studies were carried out in Toronto. Unless otherwise noted, the lipid tests were made on frozen sections of the biopsy specimens. All other tests were applied to paraffin sections. Lipids in general were colored with oil red O or Sudan black B. A solution of either reagent in propylene glycol²⁸ was used according to Wilson's trichrome method.²⁹ "Acidic" and "neutral" lipids were differentiated with Nile blue sulfate.³⁰ The former include free fatty acids and phospholipids; the latter, neutral fats, cholesterol and its esters. The acid haematein test, with its pyridine extraction control, was employed for the specific study of phospholipids³¹⁻³³ and phosphatidic acids.³⁴ Ceroid pigment³⁵ was sought in paraffin sections by coloration with oil red O or by a modification³⁶ of the Ziehl-Neelsen method. The periodic acid-Schiff's reaction³⁷ was used to demonstrate glycogen. Control sections were first incubated in a buffered solution of amylase³⁸ to remove the polysaccharide. Iron-containing pigments were identified by Gomori's modification³⁹ of Perls' test. The Feulgen reaction⁴⁰ and pyronin-methyl green staining⁴¹ supplemented, in some cases, by appropriate enzymic hydrolysis of control sections, were used when studying desoxypentose nucleic acids (DNA) and pentose nucleic acids (PNA) in the tissue sections. No special tests were performed for bile pigments.

To minimize subjective influence, all microscopic examinations were completed before the clinical data concerning each patient were made known to the observer. As explained in the footnotes to the tables of histochemical data

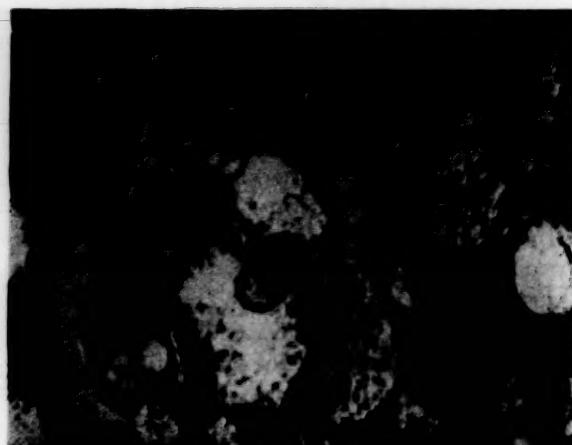


FIG. 1. Glycogen: grade 1 cytoplasmic glycogen and many glycogen-vacuolated nuclei in the parenchymal cells; no cytoplasmic fat vacuoles. Paraffin section (4 micra); periodic acid-Schiff's reaction; B and G filters, $\times 800$.

(Tables IV and VIII), the numerical values merely represent degrees of change and are, at best, only approximately quantitative. That the values for cytoplasmic glycogen and for lipids may be in general agreement with chemical determinations is suggested by the observations of Bondy, Sheldon and Evans⁴² and of Billing, Conlon, Hein and Schiff,⁴³ respectively. Our lipid estimates are based on studies of the demonstrable fats in frozen sections of tissue treated with oil red O rather than on the vacuolation evident in hematoxylin and eosin-stained paraffin sections, the former being more sensi-

tive. Lipid droplets in the cytoplasm of the cells were studied with regard to their size and number per cell, their reaction (acidic or neutral) and the location within the hepatic lobules of the cells containing them. In no instance were intranuclear lipids encountered. The grades recorded in the tables, except when otherwise specified, represent the relative amount of demonstrable lipids in a lobule of the liver. Fatty cysts or lipodiastemas, as described by Hartroft^{44,45} were sought. Their "size" was assessed on the basis not only of their dimensions compared to adjacent cells but also the number of nuclei around them. The latter is probably indicative of the number of cells contributing to their formation.

Cytoplasmic glycogen was studied in essentially the same way as the lipid droplets. The incidence of glycogen infiltration of liver cell nuclei (Figure 1) was determined by examining usually 500 to 800 nuclei and calculating the percentage containing some glycogen. During the study of sections for DNA (Figure 2) it was found that the nuclei of the connective tissue cells gave the most consistent and intense reactions. They were therefore arbitrarily chosen to represent a 4+ reaction and the liver cell nuclei were graded accordingly. No comparable "internal standard" was available for grading the PNA reactions. (Fig. 3.) So that different sections could be compared, all were processed at the same time under carefully controlled and

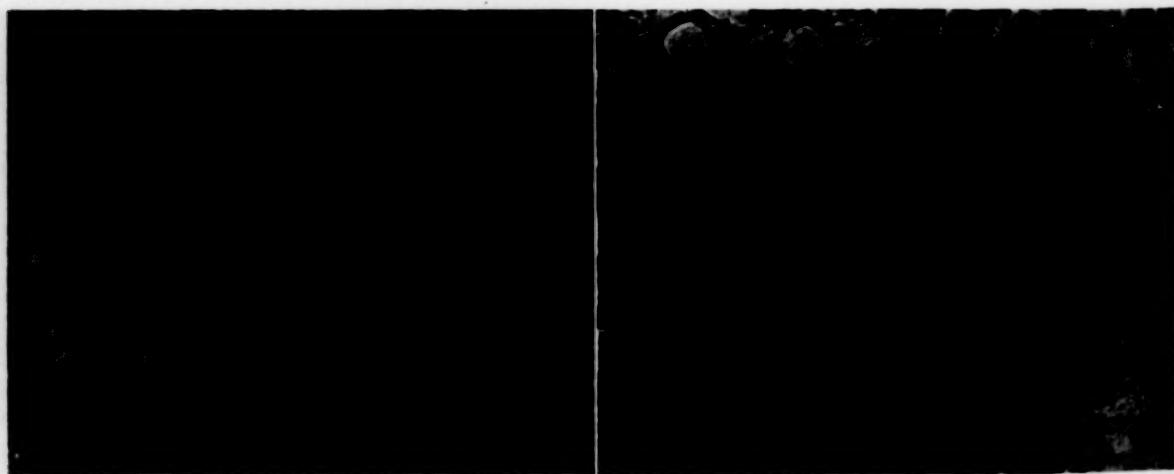


FIG. 2. Nuclear desoxypentose nucleic acids: grade 4. Paraffin section (4 micra); Feulgen reaction; B and G filters, $\times 260$.

FIG. 3. Cytoplasmic pentose nucleic acids: grade 4, as fine, uniformly distributed granules stained by pyronin. The nuclear desoxypentose nucleic acids have been stained by methyl green. Paraffin section (4 micra); pyronin and methyl green; B and G filters, $\times 230$.

standardized conditions. The distribution of iron-containing pigment was noted separately for the parenchymal and Kupffer cells.

PATIENTS WITH DIABETES MELLITUS

Clinical Data. The relevant clinical data for each of the sixteen patients are presented in Tables IA and IB. There were eight males and eight females. Their ages ranged from seventeen to seventy years. Only one (No. 9) was a juvenile diabetic. In seven (No. 1, 2, 3, 5, 8, 9 and 16) diabetes had been diagnosed from eight days to five weeks before the liver biopsy was taken; in the others, from three to twenty-seven years. If the data were available, the diets prescribed prior to the present study are recorded. Most patients have been or were obese. In four there was a history of alcoholism. Seven (No. 4, 7, 8, 11, 12, 13 and 14) had been taking insulin regularly. Two (No. 6 and 15) had taken it for only short periods following the diagnosis of their disease fifteen and six years ago, respectively. The remaining seven patients had not taken insulin before the present study. Three of them (No. 1, 2 and 5) were given no insulin prior to biopsy, although two (No. 1 and 5) received it afterward. The other four (No. 3, 9, 10 and 16) were given insulin for the first time 22, 15, 13 and 11 days, respectively, before liver biopsy. The assessed degree of "control" of the diabetes is recorded when applicable.

Listed under "Present Study" in Table I are recorded such data as the reason for admission, number of days in the hospital before biopsy, height, weight and percentage over- or underweight. The calculation of the last can be regarded as of only relative value in view of the diversity of published values for the "standard" or "desirable" body weight of a given subject.

Assessment of clinical and histologic findings indicated that the liver disease demonstrated in seven (No. 5, 6, 8, 10, 13, 14 and 16) of these sixteen patients could not or need not be causally related to their diabetes. Hepatomegaly was present in all but two of the seven. The exceptions were patient No. 6 whose portal cirrhosis, demonstrated histologically, had been unsuspected clinically, and patient No. 10 whose liver biopsy specimen revealed many sarcoid-like granulomatous lesions.

In patient No. 5 six years before the apparent onset of her diabetes the same degree of liver enlargement had been noted and portal cirrhosis had been established histologically. Patient No.

13 had a diagnosis of diabetes established for seventeen years and one of duodenal ulcer for five years. Clinical and needle biopsy findings eight months before the present study were compatible with viral hepatitis. A biopsy taken three months later revealed chronic hepatitis with some fibrosis. At the time of the present study a biopsy showed chronic hepatitis and early portal cirrhosis. The progression from acute hepatitis to early portal cirrhosis over a period of eight months is of extreme interest. Throughout this period his dietary intake was considered to be adequate. The etiology of his hepatic disease is uncertain. Although it may have been unrelated to his diabetes, the latter conceivably may have played some role in the persistence and rapid progression of the liver disease.

After careful clinical evaluation of patient No. 14, no definite cause could be found for the striking hepatomegaly. Biopsy revealed chronic, non-specific inflammation, with lesions resembling sarcoid, and degenerative, necrotic and marked fatty changes. Patient No. 16 gave a long history of choledocholithiasis with repeated episodes of mild common duct obstruction and cholangitis. At a subsequent laparotomy the terminal portion of the common bile duct was dilated and partially occluded by inspissated sludge. In patient No. 8 hepatomegaly was attributed to metastases from carcinoma of the pancreas. This was proven at autopsy eight days after the needle biopsy was taken. However, the latter did not reveal malignant tissue. The foregoing patients with hepatic disease not necessarily related to their diabetes will be considered apart from the others when attempting to assess the biochemical and other data.

In two other patients with hepatomegaly (No. 1 and 15) no conditions other than diabetes could be found to account for the slight degree of liver enlargement.

Some diabetics were in the hospital for an unrelated disease. They were studied only after this condition, if remediable (e.g., pneumonitis), had cleared and its possible hepatic effects were believed to have disappeared. Any complications of diabetes and other possible relevant diseases are tabulated. The difficulties in classifying changes such as arteriosclerosis or cataracts either as complications of diabetes or as associated disease are apparent. Included in the table are the diet and the insulin prescribed while in hospital and the average fasting blood sugar level before the biopsy was performed. Twelve

TABLE I
PATIENTS WITH DIABETES MELLITUS—CLINICAL DATA*

Case	Prior to Present Study								In Hospital	
	Sex, Age and Color	Duration ¹ of Sym- ptoms Sug- gesting D.M.	Known Duration of D.M.	Nutrition	Insulin ²	Diet Prescribed P F C	Con- trol of D.M.	Alcoholic History	Prior to Biopsy (days)	Because of
1	F, 64 W	1 y	8 d bb	Best wt. 8 y—160 lb.; lost 20 lb. during 8 m	0	0 Diet adequate	X	0	2	Symptoms of D.M.
2	M, 53 W	0	13 d bb	Usual 185 lb.; lost 30 lb. in approx. 1 m. (3½ m bb); wt. for 3½ m—155 lb.	0	0 Diet only fair; anorexia & satiety	X	0	8	For diagnosis
3	F, 64 C	0	22 d bb	45 y—95 lb.; gradu- ally gained to 223— 26 y; 22 y—210–205 lb.; 18 y—180 lb; 9 y—140 lb.; about 170 lb. since	0	0	X	0	23	Acute systemic in- fection with nervous system involvement; cause?
4	M, 60 C	0	12 y, 9 m	22 y—160 lb.; 12 y— 140–135 lb.; 11 y— 130–135 lb.; 10 y to 1 y—134–144 lb.	12 y—0 to 5 P; 11 y to 7 y—0 7 y—8 P—12P 3 y—15 P 2 y to 1 y—20 P	P—75 F—90 C—120 11 y— P—85 F—20 C—175	Fair	0	19	Pneumonitis
5	F, 68 W	0	11 d bb	7 y—184 lb.; 6 y— 144 lb.; clinical and histologic diagnosis of cirrhosis; 3 y— 182 lb.	0	Low fat diet	X	Moderate drinker	26	Cirrhosis with ascites
6	M, 64 C	0	Told he had D.M. 15 y	1 y—269 lb.	For short period after diagnosis	?	?	0	14	Pyloric obstruction
7	F, 63 C	21 y	20 y	20 y—185 lb.; 11 y— 131 lb.; following amputation left leg, 5 y—115–131 lb.	For 1 m 20 R; then 0 until 11 y; then 5–35 P; usual 10–15 P	Weighed for 9 y —type? Then: P—80–70 F—120–100 C—70–100	Poor	0	9	Infection, right foot
8	M, 40 W	4–5 m	5 w	Usual wt. 118 lb.; dropped to 94 lb. during 5 m	For 5 w—45 units “cloudy” type —?	Measured, type?	?	?	7	Carcinoma of pancreas

TABLE I (Continued)

Present Study

Height (in.)	Weight (lb.)	Ow ³ or Uw (%)	Liver ⁴	At Time of Biopsy		In Hospital		F. B. ⁵ Sugar- Mean	Biopsy Data ⁶		
				Complications of D.M.	Associated ⁸ Disease	Diet ⁶ Pre- scribed P F C	Insulin ⁷		Dietary Intake before Biopsy	Blood for Tests A.M.	Biopsy A.M.
58½	126	4 Uw	5 cm; edge round not tender; sur- face smooth	0	Cataracts	P—70 F—90 C—150	Never bb; later 40 R	268 (2)	Bft. 6:00– 6:15; lunch 10:00– 10:15	12:00– 12:06 P.M.	12:05 P.M.
71	149	15 Uw	Not enlarged	0	Carcinoma with metasta- ses to skin; probably bronchogenic	P—54 F—50 C—200 (approx.)	0	135 (1)	Approx. P—7 F—10 C—55 8:00–8:15 A.M.	11:11– 11:17	11:15
63	155	8 Ow	Not enlarged	0		P—70 F—80 C—150 Then: P—85 F—100 C—175 for 10 d bb	22 d bb, 15–30 R for 5 d; 17 d bb, 20–10 N for 15 d; 0 for 2 d bb	121 (19)	Bft. 5:00– 5:20	9:05–9:11	9:09
62½	128	7 Uw	Not enlarged	Peripheral arteriosclerosis	0	P—85 F—100 C—175	20–10 N 1 d bb—10 N	136 (16)	Bft. 5:10– 5:25	8:29–8:37	8:33
60	148	11 Ow	6 cm; edge round not tender; sur- face nodular, 9 cm.	0	Portal cirrhosis with ascites, angiomas, icterus	P—100 F—45 C—275 until 9 d bb Then: P—95 F—45 C—225	Never bb; later 15 N	133 (4)	Fstg. 19½ h	12:20– 12:30 P.M.	12:50 P.M.
?	143	?	Not enlarged	0	Carcinoma of stomach with pyloric obstruction and metastases	A11 i.v. C—38 F—0 C—75– 150 1,500 cc. blood	Not bb; later 5–35 R	112 (2)	Fstg. 10½ h	9:20–9:30	9:25
64	127	14 Uw	Not enlarged	Diabetic retinop- athy, severe; coronary heart disease; ampu- tation left leg (old) for arteriosclerosis; arteriosclerosis, right leg	0	P—70 F—80 C—150	10–70 R; then: 20–25 N 1 d bb—25 N	231 (8)	Fstg. 14 h	8:48–8:58	8:58
61½	91	31 Uw	3 cm.; edge round, firm, slightly tender; surface smooth	0	Carcinoma of pancreas; metastases to liver	P—70 F—100 C—150	35–45 R; 1 d bb—40 R	204 (4)	Fstg. 12 h	8:50–9:00	9:00

TABLE I (Continued)

Case	Sex, Age and Color	Duration ¹ of Sym- ptoms Sug- gesting D.M.	Known Duration of D.M.	Prior to Present Study				In Hospital		
				Nutrition	Insulin ²	Diet Prescribed P F C	Con- trol of D.M.	Alcoholic History	Prior to Biopsy (days)	Because of
9	M, 17 C	3 y	16 d bb	Obese since 9 y old	0	0	X	0	15	Phimosis
10	F, 30 C	0	Told B. S. high 3 y	Always obese	0	0	X	?	18	Arthritis of l. knee, l. wrist, r. ankle, and fever— 2 w; cause, ? rheum. fever ?
11	F, 33 C	4 y, 9 m	4 y, 8 m	5 y—190 lb.; 4 y, 8 m— 152 lb.; since varied from 115— 186 lb.; usual 135 lb. during 2 y	Insulin since diagnosis; usual dose 30 R & 15 P	P—70-90 F—70-100 C—150-200	Poor	Several alcoholic bouts	14	Ankle and pretibial edema; dyspnea for 1 w; diarrhea for 1½ d; cause, ?
12	M, 70 W	6 y, 7 m	6 y, 6 m	6 y—171 lb.; 6 m— 163 lb.	Usual 10 P	P—100 F—100 C—200	Poor	½ pt. whiskey/ day for many yr.	4	For investigation of anemia
13	M, 49 W	17 y, 2 m	17 y	Best wt. 134 lb.; 17 y— 119 lb.; 8 m— 103-105 lb.; 5 m— 105 lb.	First 2 y—25 P; then 35-45 P; 8 m—55 R—60 N; 5 m—60 G— 60 N	Weighed for 2 y and then estimated	Fair to poor	Moderate to heavy for 6 yr. 0 for 1½ yr.	8	Duodenal ulcer symptoms
14	F, 62 W	?	27 y	26 y—171 lb.; 6 y— 196-171 lb.; 4 y— 155 lb.; 3 y—187 lb.; 2 y—157-197 lb.; 1 y—166 lb.	25-55 R for 1 m; then 0 until 7 y; 7 y—15-30 P; 4 y— 5-20 P; 3 y—40 R & 20 P; 2 y— 10 R & 40 P; 1 y— 30-70 N or 65 G	P—59-75 F—80-120 C—120-150	Poor	0	12	Diarrhea, fever, malaise; cause— enteritis ?
15	M, 55 C	6 y	6 y	Usual adult wt. 180 lb. until 13 y; gradually dropped to 160 lb.	Some in hospital 6 y; none since	0	Poor	0	32	Gangrenous ulcer- ation of left toes
16	F, 53 W	3 y	15 d bb	4 y—140 lb.; loss of approx. 40 lb. dur- ing 3 y; 1 y—85 lb.	0	0	X	0	13	Right upper quadrant pain and vomiting

* Abbreviations: F—female; M—male; W—white; C—Negro; d—day; w—week; m—month; y—year; D.M.—diabetes mellitus; P—protein; F—fat; C—carbohydrate—in gm. 0—none; ?—unknown; X—not applicable; Fstg.—fasting; B.S.—blood sugar; bb—before biopsy.

¹ Unless other information is given, "prior to present study" is to follow all abbreviated time periods noted, i.e., 8 m indicates 8 months prior to present study.

² Doses noted in this column are the usual daily doses of insulin prescribed. R—regular; P—protamine; G—globin; N—NPH.

³ Heights recorded without shoes, and weights with patients wearing pajamas and robe weighing approximately 4 pounds. With adjustments of height and weight, % over- and underweight were calculated from tables in Duncan, G. G. Diabetes Mellitus, Principles and Treatment, pp. 268-269. Philadelphia, 1951. W. B. Saunders Co. % Ow indicates per cent overweight; % UW, underweight.

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TABLE I (Continued)

Present Study

Height (in.)	Weight (lb.)	Ow ¹ or Uw (%)	Liver ⁴	At Time of Biopsy		In Hospital		F. B. ⁵ Sugar- Mean	Biopsy Data ⁶		
				Complications of D.M.	Associated ³ Disease	Diet ⁸ Pre- scribed P F C	Insulin ⁷		Dietary Intake before Biopsy	Blood for Tests A.M.	Biopsy A.M.
60½	160	49 Ow	Not enlarged	0	Behavior problem	P—70 F—60 C—150	First 15 d bb, 15-55 R or 40 N; 1 d bb —40 N	148 (12)	Fstg. 14 h	7:48-8:15	7:50
70	277	78 Ow	Not enlarged	0	Syphilis, latent; slight cardiac enlargement	P—70 F—60 C—150	First 13 d bb, 15-30 N; 1 d bb—30 N	192 (9)	Fstg. 12 h	9:14-9:20	9:22
66	134	8 Uw	Not enlarged	Diabetic retinopathy	Emotionally very unstable; slight cardiac enlargement	P—90 F—100 C—200	5-25 R and 30-50 N; 1 d bb—60 N	163 (10)	Fstg. 12 h	8:45-8:50	8:52
66	156 (approx.)	3 Ow	Not enlarged	0	Arteriosclerotic retinopathy; multiple myeloma; renal insufficiency	P—85 F—80 C—175	10 N; 1 d bb—10 N	139 (2)	Fstg. 12 h	8:57-9:04	9:04
63½	110	22 Uw	6 cm.; edge round; slightly tender, surface smooth	Diabetic retinopathy	Duodenal ulcer, active; chronic hepatitis with fibrosis	P—103 F—88 C—255	40-80 R; 1 d bb—80 R	296 (4)	Fstg. 9 h	9:00-9:08	9:08
61	155	14 Ow	10 cm.; edge round, slight- ly tender, surface smooth; 11 cm.	Coronary heart disease; infarct, 7 y; angina; dyspnea; no rt. heart failure; periph. neuritis	Arteriosclerotic retinopathy; cataracts	P—70 F—80 C—150	70-80 G; 1 d bb—80 G	190 (9)	Fstg. 13½ h	9:10-9:20	7:35
67	116	0	3 cm.; edge sharp not tender	Peripheral arteriosclerosis; Lt. mid-thigh amputation (17 d bb)	Cataracts; arterioscler- otic and hy- pertensive retinopathy	P—70 F—80 C—150 Then: P—85 F—100 C—175 h.s. feed- ing for 15 d bb	40-50 N; then, 30-40 G; then, 10-20 N 1 d bb—10 N	190 (25)	Fstg. 12 h	8:43-8:52	8:51
56	79	39 Uw	6 cm; edge round not tender; surface smooth	0	Common duct obstruction with cholang- itis, slight	P—90 F—40 C—400	None before 11 d bb; then 15-30 N; 1 d bb—20 N; later 5 N	147 (10)	Fstg. 14 h	8:41-8:56	8:45

⁴ Levels of liver enlargement taken on inspiration. First figure noted is the measurement below right costal margin in mid-clavicular line. Second figure is below the xiphoid.

⁵ Only data considered possibly relevant noted.

⁶ Daily calories divided equally into three meals unless otherwise stated. All diets in hospital weighed.

⁷ Later doses listed only to provide information regarding insulin requirements following the biopsy.

⁸ F. B. Sugar-fasting blood sugar—mean. In parentheses are the number of days that the fasting blood sugar was determined prior to the biopsy.

Blood sugar at the time of biopsy is not included.

⁹ No insulin was given on the morning of biopsy if it was done in the fasting state. All times noted are A.M. unless otherwise stated.

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TABLE II
PATIENTS WITH DIABETES MELLITUS—LABORATORY DATA†

Case	Serum		Related to Lipid Metabolism						Related to Protein Metabolism						Related to Carbohydrate				Miscellaneous			
	Bilirubin		Serum Cholesterol			Serum Proteins			CCF			SR			Glucose Tolerance		Serum		Brom-sulfonaphthalene Retention %		Amylase	
	Direct	Total	Serum Phospholipids as mgm. %	Serum Fatty Acids mgm. %	Total	Free	Ester	Free/Total Ratio	Total	Albumin/Globulin	24 hr.	48 hr.	TT	TF	SCG	SR	Blood Sugar	Normal fast-ing 80–120 mgm. %	OGT: 100 gm. glucose by mouth; normal-normal blood sugar, at or below 120 mgm. % at 2 hr. IVGT: .5 gm./kg./½ hr. Fstg.—2 hr.	Serum Alk. Phospha-tase	Dose—5.0 mg/kg. body wt.; normal less than 5 % in 45 min.	Up to 1.2 cc. N/20 Na-OH
Normal	Up to .40 mgm. %	Up to 1.0 mgm. %	500–800 mgm. %	9–10 mgm. % P	150–250 mgm. % L.	Up to 70 % of total (50–70 % of total)	Up to 30 % of total (50–70 % of total)	7.2–16.2 mEq./L.	6–8.0 gm. % (up to .5)	0–1+ 2+	1.0–4.0	0–2+	0–2+	0–2+	0–2+	0–2+	Normal fast-ing 80–120 mgm. %	OGT: 100 gm. glucose by mouth; normal-normal blood sugar, at or below 120 mgm. % at 2 hr. IVGT: .5 gm./kg./½ hr. Fstg.—2 hr.	1.5–5.0 Bo-dan-sky units	Dose—5.0 mg/kg. body wt.; normal less than 5 % in 45 min.	Up to 1.2 cc. N/20 Na-OH	Up to 120 mg. %
1	.21	.56 (1540)	16.0	29.9	402	117	285	.29	7.84	0	0	3.0	0	0	0	0	324	2.4	10.0	44	.8
2	.43	.81 (560)	9.7	10.4	206	62	144	.30	7.04	0	0	1.0	0	0	0	0	190	OGT: —1 d bb Fstg. 114; ½ hr.—237; 2 hr.—268; 3 hr.—264	26.4	11.6*	45	5.7
3	.16	.21 (584)	8.3	11.3	245	67	178	.27	7.36	0	0	1.0	0	0	0	0	142	OGT: 10 d pb Fstg. 96; ½ hr.—197; 1 hr.—238; 2 hr.—229; 3 hr.—150	2.3	1.2	226	3.1
4	.25	.50 (930)	12.7	15.5	356	96	260	.27	8.00	0	0	2.0	0	0	0	0	154	6.0	2.5	33	.5
5	2.9	4.1 (720)	9.1	12.1	189	60	129	.32	9.44	3+	3+	6.0	3+	2+	3+	0	143	OGT: Fstg. 146; ½ hr.—237; 2 hr.—247; 3 hr.—225; 4 hr.—260; 5 hr.—176; 6 hr.—150	11.5	38.5*	33	.2
6	.43	.88 (555)	(130)	(60)	(70)	(.46)	7.6	2+	3+	3.0	0	0	0	0	142	IVGT: Fstg. 126; ½ hr.—237; 1 hr.—205; 2 hr.—178; 3 hr.—154	4.4	8.8*	35	.5
7	.16	.33 (810)	11.5	9.2	280	69	211	.33	8.16	0	0	1.0	0	0	0	0	216	2.1	4.2	56	.5
8	.43	.81 (1170)	10.5	14.2	232	118	114	.51	5.28	0	0	1.0	0	0	0	0	195	23.0	14.6*	13	Less than .1

TABLE II (Continued)

Case	Serum		Related to Lipid Metabolism				Related to Protein Metabolism*				Related to Carbohydrate				Miscellaneous						
	Bilirubin		Serum Cholesterol				Serum Proteins				Metabolism				Serum						
	Direct	Total	Serum Phospholipids	Serum Fatty Acids	Total	Free	Ester	Free/Total Ratio	Total	Albumin	24 hr.	48 hr.	Blood Sugar	Glucose Tolerance	Serum Alk. Phosphatase	Bromsulfalein Retention %	Amylase	Lipase			
9	.21	.43	(608)	(235)	(110)	(125)	(.47)	7.2	0	1.0	0	0	96	1.1	13.4	87	.6		
10	.18	.66	(608)	(173)	(63)	(110)	(.42)	6.64	0	2.5	0	2+	155	1.7	71	.9		
11	.18	.43	(640)	(142)	(42)	(98)	(.29)	7.20	0	1.5	1+	2+	65	1.2	0*	21	...		
12	.16	.37	(620)	5.6	12.6	195	74	121	.38	7.60	0	1.0	0	0	134	1.9	4.8	183	1.1	
1366	(595)	(184)	(64)	(120)	(.35)	3.72/3.42	3.72/3.88	0	4.0	0	0	243	4.6	9.9*	75	.6	
14	.6	1.1	(658)	(210)	(60)	(150)	(.29)	7.44	0	0	1.5	0	0	214	7.8	21.5	33	.5	
15	.21	.28	(628)	9.7	10.5	309	96	213	.31	6.96	0	1.0	0	0	169	3.3	5.6	83	.6	
16	1.2	1.5	(604)	9.9	12.2	403	101	302	.25	6.64	±	1+	2.5	±	2+	86	16.4	12.1*	36	.1

† Abbreviations: . . . tests not performed; bb—before biopsy; pb—after biopsy; hr.—hour; CCF—cephalin cholesterol flocculation; TT—thymol turbidity; TF—thymol flocculation; SCG—serum colloidal gold; SR—scarlet red; OGTT—oral glucose tolerance; IVGT—intravenous glucose tolerance.

* Determinations indicated by parentheses in tests "Related to Lipid Metabolism" were performed at the Graduate Hospital. Those without parentheses were performed in the laboratory of Dr. I. J. Pincus, Jefferson Medical College.

† The results of serum protein studies performed by paper electrophoresis and by the Tiselius method in some of these patients are described in the text.

* In all cases but those indicated by *, the Bromsulfalein test was performed within three days before or after the biopsy. In the remaining cases this test was performed as follows:

Case No. 3—4 d bb
6—11 d bb
8—4 d bb
5—7 d pb
13—6 d bb
16—9 d bb

patients were investigated after fasting from nine to nineteen and one-half (usually twelve to fourteen) hours. Four were studied after they had eaten. Their dietary intakes and the intervals before biopsy are recorded. The entire procedure of taking blood and aspirating liver tissue was performed within ten minutes in twelve patients and within 15 to 105 minutes in the other four (No. 5, 9, 14 and 16).

Laboratory Studies. The results of the various biochemical studies are recorded in Table II. Excluded from analysis are the results in nine patients (No. 2, 5, 6, 8, 10, 12, 13, 14 and 16) with disease causally unrelated or probably unrelated to diabetes.

Serum bilirubin: Direct reacting and total serum bilirubin values were normal.

Serum lipids: When assessing serum lipid values it is advisable to attach little importance to any slight deviations from the normal ranges given in Table II. There were no significant abnormalities that could not be explained by the non-fasting state or by complicating diseases.

Serum proteins: Abnormalities in the serum proteins were present in six of the seven diabetic patients. In five the serum globulins were above normal. The serum albumin fraction was diminished in all but patients 7 and 9. Because the method used for fractionating serum proteins may not give adequate separation of albumin and globulins, it is likely that little significance should be attached to the minor changes in serum albumin and globulin values noted in Table II. It is of interest, however, that the albumin/globulin ratio was described as abnormal in 110 of 380 diabetic patients studied by Leevy, Ryan and Fineberg.⁴

Paper electrophoretic studies were performed in five patients (No. 1, 2, 3, 7 and 12). An unusual fraction with a mobility intermediate between that of alpha₂- and beta globulins was noted in one case (No. 2). The gamma globulin pattern appeared to be increased moderately in two cases (No. 2 and 3) and markedly in one (No. 12). The peaks for albumin were decreased in one (No. 7) and possibly in two others (No. 2 and 3). The precipitation method revealed abnormalities in three of the four cases in which the paper electrophoretic studies showed alterations. The results of both precipitation and electrophoretic studies were normal for patient No. 1.

The high incidence of serum protein abnor-

malities in this small group of hospitalized diabetics in whom no marked liver disease was demonstrable is of interest. Before ascribing these changes to the diabetic state it is important to recognize the possibility that other factors may have been responsible in some instances. The disease present at the time of admission and from which the patient had clinically recovered by the time of the biopsy may have been concerned. The role of associated diseases should be considered in some cases; for example, multiple myeloma in patient No. 12.

"Turbidity" and "flocculation" tests: The results of all of these tests were normal except for the scarlet red test which was abnormal in patient No. 11. Zimmerman and his associates⁴⁶ also found a low incidence of abnormal cephalin-cholesterol flocculation tests in diabetic patients but Leevy, Ryan and Fineberg⁴ found it abnormal in 30 per cent of 380 patients.

Blood glucose: In the four patients (No. 1, 2, 3 and 4) who were not fasting and in three (No. 7, 12 and 15) of those who were fasting, some degree of hyperglycemia existed at the time of biopsy.

Serum alkaline phosphatase: This was slightly increased in patient No. 4 but normal in the other patients.

Bromsulfalein retention: Significant retention of bromsulfalein was noted in two (No. 1 and 9) of seven patients in whom disease causally unrelated or probably unrelated to diabetes was excluded. Zimmerman, MacMurray, Rappaport and Alpert⁴⁶ also found impaired bromsulfalein excretion in twelve of twenty-eight patients with uncomplicated diabetes mellitus, while Leevy and his co-workers⁴ found this test to be abnormal in approximately 58 per cent of 380 patients with diabetes.

Serum amylase and lipase: Serum amylase and lipase values were both elevated in one patient (No. 3).

Histologic Studies. In the previously mentioned seven diabetic patients with hepatic disease causally unrelated or probably unrelated to their diabetes (No. 5, 6, 8, 10, 13, 14 and 16) as well as in seven of the remaining nine cases, there were other less striking yet abnormal findings. These consisted of focal lesions (Fig. 4) with or without mononuclear cell infiltration, and various degenerative cytoplasmic and nuclear changes. (Table III.) The focal lesions were interpreted as being necrotic in origin. In some

the cells appeared to be undergoing resorption, in others they were atrophic. Possibly, some of these lesions were artifacts. They had no definite zonal distribution. In some sections they were rare; in others, relatively numerous. Connective tissue and reticulin stains usually revealed collapse of the reticular network where parenchymal cells had disappeared. Some of these hepatic lesions resemble those found by Zelman⁴⁷ in obese patients. The cytoplasmic changes included loss of granularity, loss of cell outline, "smudging" or, less often, intense eosinophilia. In two patients who partook of appreciable amounts of alcohol, hyaline masses ("Mallory bodies") were noted in the cytoplasm of some degenerating hepatic cells. The most prominent nuclear change was glycogen vacuolation. Pyknosis, occasionally chromatolysis and, rarely, karyorrhexis were also noted.

In the diabetic patients with concomitant cirrhosis it is impossible to decide what role the diabetes itself may have played in initiation of the necrotic and degenerative changes which were noted. However, in the case with secondary biliary cirrhosis (No. 16) the incidence of the focal lesions and the degree of the degeneration were greater than might be expected from the slight changes due to extrahepatic biliary tract disease. The lesions noted in the group of diabetics without cirrhosis could not be attributed to any other cause. They might be related in some as yet unidentified manner to the metabolic disturbances extant in diabetes.

To extend our observations on the focal lesions and degenerative changes found in the liver biopsies of diabetic patients, the liver biopsy material in the Department of Pathology, Graduate Hospital, was reviewed. Hematoxylin and eosin-stained sections of tissue from three diabetic patients without other liver disease such as cirrhosis were available. In two of these focal lesions and degenerative changes were prominent. Both patients were in fair diabetic control. Despite poor diabetic control prior to and during hospitalization, no pathologic changes were noted in the liver of one patient.

That the focal lesions are not solely or primarily due to diabetes mellitus is shown by their occurrence in association with other disease. Such lesions were found in three of our seven patients with cirrhosis without diabetes (No. 21, 22 and 23) and in six of the patients with other liver diseases. Swife, Kessler and Lisa⁴⁸ noted similar focal lesions in twelve of their sixty-three

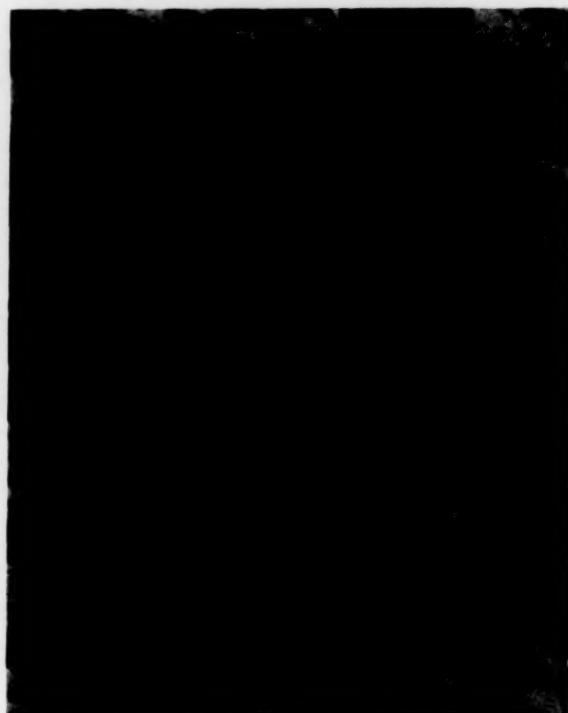


FIG. 4. Focal lesion: a small circumscribed lesion involving the hepatic parenchyma and consisting of shrunken, degenerated and necrotic cells with pyknotic nuclei. Paraffin section (7 micra); hematoxylin and eosin; B and G filters, $\times 190$.

cases of alcoholic cirrhosis. They found that the lesions were inconsistent in their occurrence and apparently unrelated to other histologic abnormalities or to any clinical features. Among twenty obese but otherwise normal persons, Zelman⁴⁷ found significant numbers of these focal necrotic lesions in one and lesser amounts in thirteen persons.

In view of the great functional reserve of the liver, these slight, non-specific changes may prove to be insignificant. If they were diffuse or prominent, however, they might be at least partly responsible for the impairment of hepatic function sometimes found in diabetic patients. However, in our cases there was no correlation between the incidence of the focal lesions and the extent of bromsulfalein retention or of abnormalities in other liver function tests. Nor was the incidence of these focal changes related to clinical or histochemical findings.

In some of our cases regional and uneven variations in the distribution of parenchymal fatty cysts, histochemically demonstrable lipids and nuclear vacuolation were found in the same or different portions of liver specimens obtained by needle biopsy. Auxiliary studies⁴⁹ demon-

strated that appreciable variations in histochemical findings may sometimes occur between different biopsy specimens of the same liver, and possibly lead to erroneous conclusions.

Histochemical Studies. The results of histochemical studies on liver biopsy specimens from

the diabetic patients are presented in Table IV. The data for lipids are based on the tests with oil red O, Sudan black B and Nile blue sulfate. The acid haematein method revealed phospholipids principally in the mitochondria (Figs. 5, 6 and 7) but in none of the intracellular fat

TABLE III
PATIENTS WITH DIABETES MELLITUS—HISTOLOGIC OBSERVATIONS¹

Case No.	Histologic Findings ²	Portal Cirrhosis	Portal		Focal Lesions			Paren. Cyto. Degen.	Nuclei		Kupffer Cell Prominence
			Fibrosis	Mononuc. Infiltr.	Degree	Distribu-tion	Mononuc. Infiltr.		Pyknosis	Vacuola-tion or Ballooning	
1.	Focal lesions; parenchymal cell degeneration	0	0	0	1	Diffuse	+	2	0	4	0
2.	Focal lesions; parenchymal cell degeneration; portal fibrosis and mononuclear cell infiltration	0	1	2	1	Diffuse	0	1	1	3	0
3.	Focal lesions; portal fibrosis	0	1	0	1	Diffuse	+ and 0	0	0	1	0
4.	Focal lesions; parenchymal cell degeneration	0	0	0	1	Diffuse	+ and 0	2	0	0	0
5.	Portal cirrhosis; focal lesions; parenchymal cell degeneration	4	—	1	3	Diffuse	+ and 0	4	0	1	0
6.	Portal cirrhosis; focal lesions; parenchymal cell degeneration	2	—	1	3	Diffuse	+	2	1	tr.	0
7.	Focal lesions; parenchymal cell degeneration	0	0	0	1	Diffuse	0	1	0	0	0
8.	Focal lesions	0	0	0	1	Diffuse	+	0	0	tr.	0
9.	"Normal"	0	0	0	0	—	—	0	0	4	0
10.	Focal lesions; parenchymal cell degeneration; portal fibrosis and mononuclear cell infiltration; sarcoid-like granulomatous lesions	0	1	1	3	Diffuse	+	1	0	2	0
11.	Focal lesions; parenchymal cell degeneration	0	0	0	1	Diffuse	0	2	0	0	0
12.	Focal lesions; parenchymal cell degeneration	0	0	0	1	Diffuse	0	2	0	tr.	1
13.	Chronic hepatitis with early portal cirrhosis	1	3	4	2	Diffuse-marked central	+	3	0	1	0
14.	Focal lesions; parenchymal cell degeneration; portal mononuclear cell infiltration; sarcoid-like granulomatous lesions	0	0	3	3	Diffuse	+	4	0	3	1
15.	"Normal"	0	0	0	0	—	0	0	0	tr.	1
16.	Secondary biliary cirrhosis; focal lesions; parenchymal cell degeneration	0	2	1	3	Diffuse	+ and 0	3	0	2	0

¹ Explanation of Terms and Abbreviations: Portal cirrhosis—classic, with definite pseudolobular formation. Portal fibrosis—increase in connective tissue of portal areas but with no pseudolobule formation. Focal lesions—lesions considered to be of necrotic origin. Mononuc. infiltr.—mononuclear cell infiltration of portal areas. Paren. Cyto. Degen.—cytoplasmic degeneration of parenchymal cells. Diffuse—no special zonal distribution. Central—about central veins.

² Grading of observations ranges from 0 to 4 and also includes within the interval 0–1, tr. (trace). 0, not present; 1, definite but slight; 2, moderate; 3, quite marked; 4, severe. + = present; — = not applicable.

³ Summary of Main Histologic Findings, ungraded. Lipid changes and nuclear vacuolation (glycogen) are recorded with the histochemical observations in Table IV.

TABLE IV
PATIENTS WITH DIABETES MELLITUS—HISTOCHEMICAL OBSERVATIONS

Case No.	Histologic Observations ¹	Histochemical Observations ²							
		Lipids ³		Glycogen ⁴		Nucleic Acids ⁵		Iron Pigment	
		Cytoplasmic Droplets	Cysts	Cytoplasmic	Nuclear	Nucl. DNA	Cyto. PNA	Parenchymal Cells	Kupffer Cells
1.	Focal lesions; parenchymal cell degeneration	4 med., neut. genl.	2 lg., neut. and cryst. scat., no trabec.	3½ to 3½ mkd. varn.	2 to 30 %	1	2	v. f. tr.	3
2.	Focal lesions; parenchymal cell degeneration; portal fibrosis and mononuclear cell infiltration	f. tr. v. sm., ac., cent.	tr. lg., ac., cent.	4 unif.	12	2	3	1	3
3.	Focal lesions; portal fibrosis	0	0	4 unif.	5	2	...	0	0
4.	Focal lesions; parenchymal cell degeneration	0	0	4 unif.	0	2	1	0	0
5.	Portal cirrhosis; focal lesions; parenchymal cell degeneration	3 med. and lg., neut., genl.	2 sm., neut., scat., occas. trabec.	4 unif.	1	2	4	0	0
6.	Portal cirrhosis; focal lesions; parenchymal cell degeneration	tr. v. sm., occas. sm., neut., scat.	0	2 unif.	0.6	1	1	f. tr.	0
7.	Focal lesions; parenchymal cell degeneration	1 sm., ac., occas. neut., port.	0	3 mod. varn.	0	1½	...	0	0
8.	Focal lesions	3 sm., ac., genl. 1 lg., neut., port.	tr. med., neut., scat.	3 decr. port.	0.6	1	1½	1	3
9.	"Normal"	tr. v. sm., neut. cent.	3 med., neut., genl.	3 unif.	26	...	1	0	1
10.	Focal lesions; parenchymal cell degeneration; portal fibrosis and infiltration; granulomatous lesions	2 sm. and med., neut., occas. ac., genl.	2 med. and lg. neut. and cryst., scat., no trabec.	2 mkd. varn.	3	2	3	0	0
11.	Focal lesions; parenchymal cell degeneration	3 med., neut., cent.	tr. sm., neut. cent.	3 unif.	0	2	3	0	0
12.	Focal lesions; parenchymal cell degeneration	1 v. sm., neut., port.	1 med., neut., port.	2 unif.	0.5	1	2	1	3
13.	Chronic hepatitis with portal cirrhosis	2 sm., neut., port.	0	4 unif.	2	1	1	tr.	0
14.	Focal lesions; parenchymal cell degeneration; portal mononuclear cell infiltration; sarcoid-like granulomatous lesions	3 med., neut., genl.	4 med. and lg., neut. genl.	4 unif.	7	3	1½	0	0
15.	"Normal"	1 fine, ac. and neut., genl.	tr. sm., ac., trabec.	1½ unif.	0.4	1	1	0	1
16.	Secondary biliary cirrhosis; focal lesions; parenchymal cell degeneration	3 sm. to lg., neut. genl.	0	1 unif.	3	1	tr.	0	tr.

¹ Summary of histological observations: details are recorded in Table III.

² Grading of observations for lipids, glycogen, nucleic acids and iron pigment: ranges from 0 to 4 and includes within the interval 0-1, v. f. tr. (very faint trace), f. tr. (faint trace), and tr. (trace).

³ Lipids: Separate data are given for cytoplasmic fat droplets and for fatty cysts as follows:

Size: v. sm.—very small; sm.—small; med.—medium; lg.—large.

Reaction: ac.—acidic; neut.—neutral; cryst.—crystals also present.

Distribution within hepatic lobule: genl.—general; port.—periportal; cent.—central; scat.—scattered; trabec.—intratrabecular.

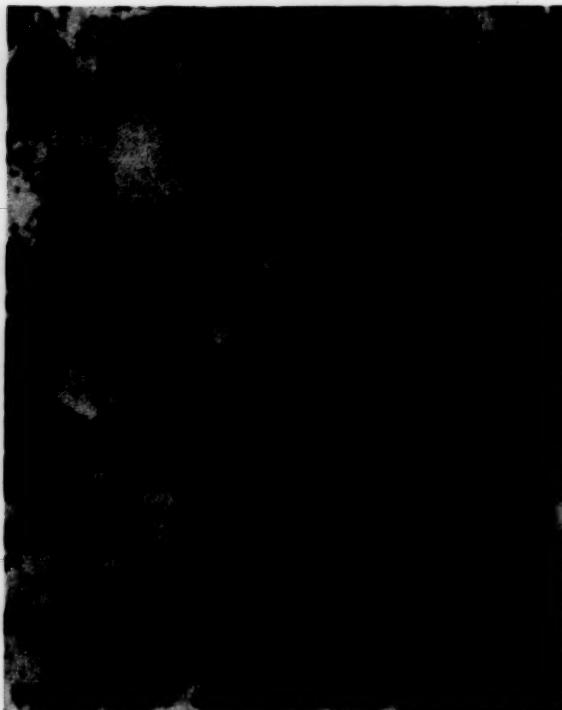
⁴ Glycogen:

Cytoplasmic: Distribution: unif.—uniform; varn.—variation; mkd.—marked; mod.—moderate; decr. port.—decreased in periportal regions.

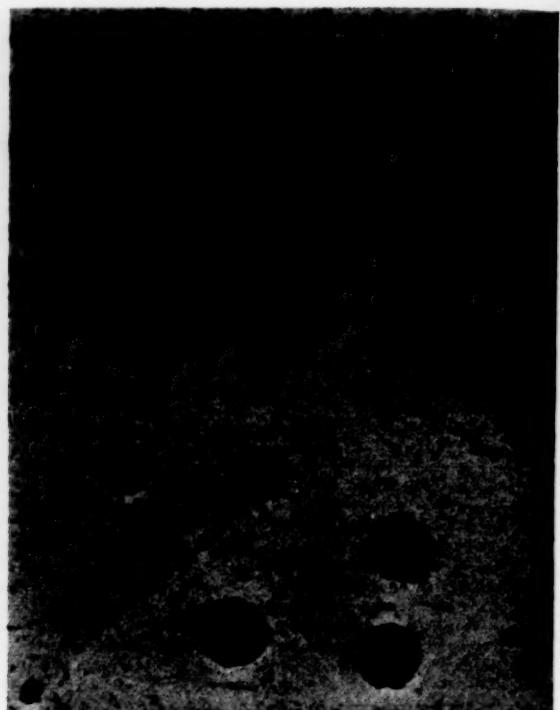
Nuclear: Percentage of nuclei containing some glycogen.

⁵ Nucl. DNA—Nuclear desoxyribose nucleic acid.

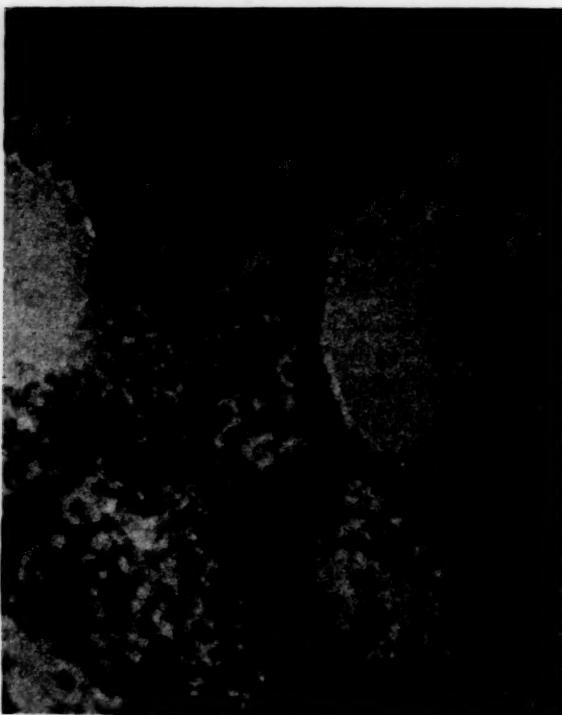
Cyto. PNA—Cytoplasmic pentose nucleic acid.



5



6



7

Figs. 5, 6 and 7. Phospholipids: in the parenchymal cells, histochemically demonstrable phospholipids and phosphatidic acids occur almost exclusively in the mitochondria where they are stained black by the acid haematein test (tiny rodlets in Figure 5) and unstained in the pyridine extraction control. (Fig. 6.) When present, cytoplasmic lipid droplets displace the mitochondria. (Fig. 7.) Note that the parenchymal cell nuclei are unstained in the acid haematein test (Figs. 5 and 7) but are evident in the pyridine extraction control (Fig. 6) while erythrocytes are stained black (dense black masses best seen in Figure 7). Neither staining reaction is indicative of phospholipids. Frozen sections (5 micra); Figures 5 and 7, acid haematein test; Figure 6, pyridine extraction control; G filter. Figure 5, $\times 490$; Figure 6, $\times 550$; Figure 7, $\times 1040$.

droplets. Apart from mitochondrial displacement by such droplets, no abnormalities in distribution or appreciable quantitative variations in the histochemically demonstrable phospholipids and phosphatidic acids were noted. It is probable, therefore, that the "acidic" lipids revealed by Nile blue sulfate were mostly fatty acids. Ceroid was not found in any case.

Despite the carefully controlled conditions under which these patients were studied, diabetes mellitus was not the sole factor possibly affecting the hepatic cells in most of our patients. Insulin administration, fasting, state of nutrition, alcoholism, obesity and other concurrent diseases have to be considered. The histochemical observations reflect only the net effect of all such factors and therefore their significance is often difficult to assess. In order that such assessment may be as accurate as possible, every possibly relevant detail concerning factors which might influence the liver cells should be noted.

In only one patient (No. 1) were a minimum of these factors active at the time of biopsy. She had moderately severe diabetes, was not fasting, was not appreciably underweight, gave no history of alcoholism and had no associated disease possibly affecting her liver. Neutral lipids had accumulated as numerous medium-sized droplets in most of the parenchymal cells and as scattered, large cysts, some of which contained fine crystals. (Fig. 8.) The cells surrounding the central veins had little glycogen in their remaining cytoplasm but nearly a third of them had considerable amounts in their nuclei. The distribution of glycogen was reversed in the periportal cells. Both nuclear DNA and cytoplasmic PNA were diminished in the parenchymal cells. The Kupffer cells were filled with hemosiderin.

Lipids: Although lipids have been long known to accumulate in the livers of diabetic patients,⁵⁰ their nature and site of initial deposition remain unsettled. The case just described demonstrates that in moderately severe diabetes, untreated with insulin, neutral lipids may appear in all the hepatic cells. Very few lipid droplets were found in the liver of another diabetic who had been fasting and received no insulin before or after biopsy (No. 2), but fatty cysts attested to the previous accumulation of much larger amounts of fat which may have been mobilized during an earlier period of undernutrition. Generalized carcinomatosis, too, may have affected his liver lipids. No demonstrable fats were found

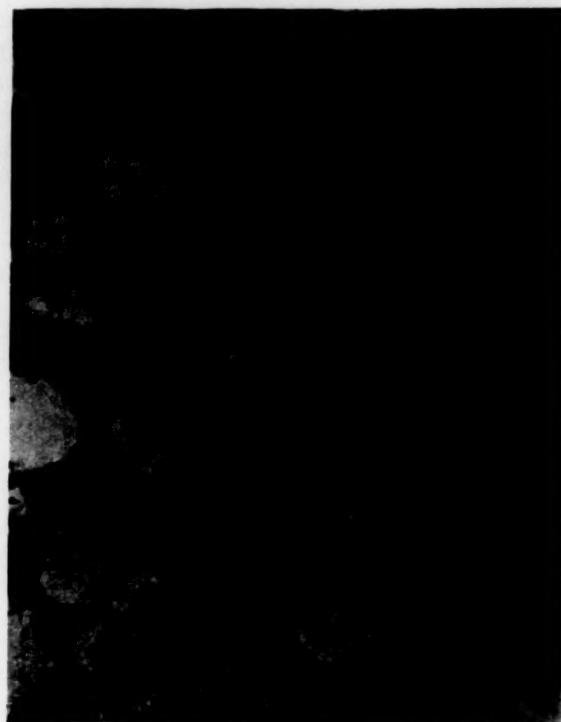


FIG. 8. Lipids: neutral fats occurring as many medium sized droplets (dark grey or black in the photomicrograph) and as large parenchymal fatty cysts some of which contain fine lipid crystals. Frozen section (7 micra); oil red O, hematoxylin and light green; B filter; X 265.

in the livers of the other two non-fasting patients who had been receiving insulin up to one (No. 4) or two (No. 3) days before the biopsy. Such absence might be attributable to either insulin administration or the mildness of their diabetes. No other significant factors were apparent.

Unlike the preceding four patients, the other twelve were fasting when their liver biopsies were taken. All but two (No. 5 and 6) had received insulin, many were appreciably over- or underweight, some had liver disease and others had associated diseases which might have affected their livers. Of the two patients who had received no insulin, the one with the shorter history of diabetes but with advanced portal cirrhosis (No. 5) exhibited marked accumulation of neutral lipids as cytoplasmic droplets and small cysts in the liver. Despite their possible pathogenetic role, only a few of the latter occurred within the fibrous trabeculae. Only negligible amounts of neutral lipids were noted in the liver of the other patient who had had diabetes for a longer time (No. 6). This finding may be related to his being poorly nourished and having pyloric obstruction due to carcinoma.

In the livers of all ten diabetics who had received insulin and were fasting before their biopsies were taken neutral fats were present as cytoplasmic droplets, ranging from mere traces to considerable quantities in the different cases. The distribution of these droplets within the hepatic lobules varied, being general in four patients (No. 10, 14 to 16), only centrilobular in two (No. 9 and 11) and periportal in four (No. 7, 8, 12 and 13), probably reflecting the variety of possible influencing factors. In addition, in eight cases (No. 8 to 12, 14 and 15), neutral lipids were found in fatty cysts and, in four cases (No. 7, 8, 10 and 15), acidic lipid droplets were also present. Fat was noted in Kupffer cells in only a few cases, in contrast to Chiari's observations.⁵¹ He reported that this was a frequent finding possibly related to the severity of the diabetes.

Fatty cysts are evidence of prolonged fat accumulation.^{44,45} They disappear slowly during specific therapy whereas the cytoplasmic fat droplets go comparatively quickly. The findings in patient No. 9 may be indicative of effective therapy. Only fatty cysts remained as stigmata of a previously very fatty liver which may have been related to his being obese for eight years or, more likely, to his being a juvenile diabetic. Only minimal chronic fatty changes were apparent in the liver of patient No. 11 who had diabetes for nearly five years. The marked accumulation of lipid droplets may be persisting evidence of her previously poorly controlled disease or of her intake of alcohol. She was not obese. No cysts were noted in the liver of patient No. 7 whose diabetes had been poorly controlled for twenty years.

In the parenchymal cells of the livers of three of the four diabetic patients who were studied while not in the fasting state, there were no or only negligible amounts of histochemically demonstrable lipids. On the other hand, appreciable quantities of these had accumulated in the cells of eight of the twelve diabetics who were studied while fasting. In consideration of the many experimental observations, it is probable that such changes, especially when slight and periportal, may be at least partly attributed to fasting. However, when the accumulation of lipids is severe and of long duration as evidenced, for example, by fatty cysts and crystals of lipids, the fatty changes are undoubtedly related to the diabetes or some cause other than the fasting. Our findings sug-

gest that changes in the histochemically demonstrable hepatic lipids may be as dynamic in human diabetics as they are in experimental animals and, further, that the neutral fats are the ones most extensively involved. That there is no demonstrable alteration in the phospholipids is in agreement with the analyses reported by Halliday.⁵² The question of the initial site of appearance of lipid droplets in diabetic livers remains unsettled.

Glycogen: Although carbohydrate metabolism is profoundly altered in diabetes mellitus, relatively little is known histochemically about this aspect of the disease. Glucose, for example, cannot be localized even in frozen-dried tissues. The method introduced by Okamoto, Kadota and Aoyama⁵³ holds some promise⁵⁴ but could not be employed in the present investigation. Glycogen, however, can be readily studied. For this purpose the liver biopsy technic is especially valuable because rapid *postmortem* hydrolysis can be avoided.

The changes described in patient No. 1 are typical of those found in untreated diabetics.⁵⁵ The cytoplasmic glycogen was most abundant in the periportal cells and least plentiful or even lacking in the central ones. Intracellular deposits of glycogen were most often noted in the cells with the least cytoplasmic glycogen, a reciprocal relationship, existing. In the livers of the other three patients who were not fasting when their biopsies were taken (No. 2 to 4), there was abundant glycogen uniformly distributed throughout all cells. In all but two (No. 12 and 14) of the twelve fasting subjects lesser amounts of cytoplasmic glycogen were found. In those cases in which this was not uniformly distributed it tended to be most plentiful in the periportal cells. Warren and LeCompte⁵⁶ have attributed this phenomenon to the greater synthesis of glycogen by the cells "best supplied by nutrient and oxygen." In none of our cases was there any evidence of antagonism between the deposition of glycogen and the accumulation of fat within the cytoplasm of the parenchymal cells.

Intranuclear glycogen deposition, giving rise to vacuolation, occurred to some degree in thirteen of the sixteen diabetic patients. Our observed total incidence of this change (Table V) is somewhat greater than that reported by Zimmerman and his associates⁵⁷—the increase being in those grouped as "minimal" or "moderate." Our data, like theirs, reveal no significant

relationship between this nuclear abnormality and such factors as the average fasting blood sugar level or the clinical status of the diabetes. As also noted by Chipp and Duff,⁶⁶ it would appear that nuclear vacuolation is not primarily associated with hyperglycemia. It also occurs in diseases other than diabetes.⁶⁶ Its ultimate cause remains unknown. Eger and Klarner⁶⁷ suggested that glycogen is formed within the nucleus and then stored in the cytoplasm. On this basis Warren and LeCompte⁶⁸ have suggested that nuclear deposition of glycogen may be evidence of altered glycogenesis within the nucleus or altered permeability of the nuclear membrane to glycogen. In diabetes, at least, such deposition is an example of pathologic storage occurring in a primarily metabolic disease.

Desoxypentose nucleic acids: Nuclear DNA was investigated in fifteen of the sixteen diabetic patients. Generally, there was no marked variation between the nuclei in any given case nor between patients. Slightly decreased amounts of DNA were noted in seven cases (No. 1, 6, 8, 12, 13, 15 and 16). The reliability of these observations and of those on PNA may be questioned since ethanol may not be a satisfactory fixative for nucleic acids.⁶⁸ Neither DNA nor PNA appears to have been previously studied in liver biopsies from diabetic patients.

Pentose nucleic acids: Cytoplasmic PNA was studied in fourteen of the diabetic patients. Slight to marked decreases in this constituent were noted in all but four cases (No. 2, 5, 10 and 11). When large amounts of PNA were present the pyronin-stained granules were distributed uniformly throughout the cytoplasm of each cell. (Fig. 5.) The greater the reduction in PNA, the greater the tendency for these granules to be concentrated around the nuclei or near the bile canaliculi. This distribution did not appear to be the result of mechanical displacement by fat droplets or glycogen. It resembles that seen during protein starvation.⁶⁹ Usually, in those cases in which decreased granularity of the cytoplasm was noted histologically, reduction in PNA was observed histochemically.

The relation between PNA and protein synthesis has been reviewed by Greenstein⁶⁰ and,

from a histochemical viewpoint, by Dempsey and Wislocki.⁶¹ PNA may be an index of cellular protein content. It may be, therefore, that PNA depletion is related to the protein catabolism occurring in diabetes but, in our cases, it was unrelated to insulin administration. Protein

TABLE V
OCCURRENCE OF GLYCOCEN-CONTAINING NUCLEI

Data of Zimmerman et al.		Data for This Study	
Degree	Number	Incidence	Number
Severe.....	6 (21%)	> 7%	3 (19%)
Moderate.....	5 (18%)	2-7%	5 (31%)
Minimal.....	5 (18%)	< 2%	5 (31%)
Absent.....	12 (42%)	0	3 (19%)

starvation may have been a factor, especially in patient No. 6, with pyloric obstruction who had lost 126 pounds in one year; in patient No. 8, with metastasizing carcinoma; and in patients No. 15 and 16. In most though not in all instances some co-existent disease, for example malignancy, may have been responsible for some of the decrease in cytoplasmic PNA.

Iron pigments: Some hemosiderin was found in the parenchymal cells of six of the sixteen diabetics (No. 1, 2, 6, 8, 12 and 13). Three (No. 6, 8 and 13) of these six patients had hepatic disease unrelated or probably unrelated to diabetes. In all but one case (No. 2) the presence of hemosiderin in parenchymal cells was associated with at least slight accumulation of fat although there was no quantitative correlation. Zimmerman, MacMurray, Rappaport and Alpert⁶⁸ also found hemosiderin in the hepatic cells in eight of twenty-eight diabetic patients and these eight patients were among the fourteen who had some fat in their livers. The origin and significance of this iron-containing pigment remains obscure although it may be evidence of previous hepatocellular necrosis.

Hemosiderin was found in the Kupffer cells of seven of our sixteen diabetic patients (No. 1, 2, 8, 9, 12, 15 and 16) but could not be correlated with previous transfusions.

II. Portal Cirrhosis and Other Liver Diseases

Clinical Data. Seven patients with portal cirrhosis were studied in the same manner as the diabetics. Relevant clinical data are presented in Table VI. From these and the biochemical and histologic findings it was considered that five patients (No. 17 to 21) had portal cirrhosis of "marked" degree, one (No. 22) "moderate" and one (No. 23) "mild."

Laboratory Studies. The results of the laboratory tests are presented in Table VII.

Serum bilirubin: At the time of biopsy, in all but one patient (No. 20) the direct reacting serum bilirubin levels were elevated, the highest being 2.4 mg. per cent. Only two patients (No. 17 and 20) had normal total serum bilirubin values. The highest in the remaining cases was 3.1 mg. per cent.

Serum lipids: The total serum lipid levels were normal in all but one patient (No. 22) who also showed significant elevations of serum phospholipids, fatty acids, total serum cholesterol and the free:total cholesterol ratio. The total serum cholesterol level was elevated in one other case (No. 23) and the free:total cholesterol ratio in another (No. 17). Serum phospholipid and fatty acid studies were performed in only two other patients and revealed no significant changes.

Serum proteins: The total serum protein concentrations were within the normal range in all cases. However, the albumin and globulin values show that the serum proteins were abnormal in four (No. 18, 19, 20 and 21) of the seven patients. Paper electrophoretic studies of the serum from patient No. 21, who had recovered from hepatic coma, revealed the presence of a fraction with a mobility between the alpha₂- and beta-globulins. The gamma-globulin pattern suggested an increase in this fraction. Electrophoretic analysis of the serum from one of the patients with "marked" portal cirrhosis (No. 20) gave the following percentages: albumin, 41.5; globulins; alpha₁, 5.7, alpha₂, 11.8, beta, 16.3 and gamma, 24.7, indicating a relative increase in most of the globulin fractions and a decrease in the albumin fraction.

"Flocculation" and "turbidity" tests: When the liver biopsies were taken, the result of the forty-eight-hour cephalin-cholesterol flocculation test was abnormal in only two (No. 18 and 19) of the

seven patients. In one of these (No. 18) the thymol flocculation, serum colloidal gold and scarlet red tests were also abnormal, and the thymol turbidity determination equivocal. For the other five cases the results of all tests were normal.

Blood sugar and glucose tolerance: Hyperglycemia (224 mg. per cent) was noted in only the patient who was not fasting at the time of biopsy (No. 21). His fasting blood sugar values and those of the other six patients were normal. Some impairment of glucose tolerance was noted in all four patients (No. 18, 19, 21 and 22) in whom this was studied.

Serum alkaline phosphatase: The serum alkaline phosphatase level was at the upper limit of normal in one patient (No. 21), elevated in another (No. 22) but normal in the rest.

Bromsulfalein retention: Impaired bromsulfalein excretion was found in all seven patients but to only a minor degree in one (No. 23).

Serum amylase and lipase: Abnormal serum amylase and lipase levels which persisted for several days were present in two patients (No. 19 and 21). In the other six the values were normal.

Histologic Studies. The characteristic findings of Laennec's cirrhosis were present to some degree in all seven cases. The relevant histologic findings are briefly recorded in the first column of Table VIII.

No fatty cysts were found in the livers of three of the cirrhotic patients (No. 17, 18 and 21), only intratrabecular ones (Fig. 9) in two (No. 19 and 20) and only parenchymal ones (Fig. 8) in the other two (No. 22 and 23), yet all gave significant histories of alcoholism and most (No. 17 to 21) had "marked" cirrhosis. Both types of cysts were found in the liver of one of the diabetic patients who had portal cirrhosis (No. 5) but none in that of the other (No. 6) or in those with chronic hepatitis and slight portal cirrhosis (No. 13) or slight biliary cirrhosis (No. 16).

After studying autopsy specimens from a large number of cirrhotic patients, Hartroft⁴⁵ concluded that intratrabecular fatty cysts suggest that the cirrhosis is the result of "a previous period of excessive fat storage." He noted that parenchymal fatty cysts were sometimes encountered "when the clinical diagnosis had

indicated that the cirrhotic lesions were not the sequel to damaging degrees of fat accumulation." It would be most difficult clinically to differentiate our seven cases with respect to which livers might have had antecedent fatty changes. Of particular interest was patient No. 20 in whom a liver biopsy taken five months before the study had revealed both parenchymal and intratrabecular fatty cysts. He denied the ingestion of alcohol during the interval and had had a nutritious diet. The present biopsy showed only intratrabecular cysts. This finding strongly supports Hartroft's suggestion. However, there is no greater indication that a fatty liver preceded the cirrhosis in the other patient with intratrabecular cysts (No. 19) than in the remaining cases. Although these observations concern only a few cases, they may indicate that fatty cysts may not have the same significance in needle biopsy specimens. On the other hand, the absence of fatty cysts in the livers of the three patients with biliary cirrhosis noted below, a condition unlikely to be the sequel of fatty liver, favors Hartroft's interpretation.

That there may be no correlation between the turbidity or flocculation tests and the histologic findings was well demonstrated in this study. The results of all the tests were normal in five of the seven patients from whom blood and liver biopsy specimens were taken simultaneously. Nevertheless, there were strikingly abnormal histologic findings in all cases. In two patients with abnormal turbidity and flocculation tests there was no hepatocellular necrosis but diffuse parenchymal cell degeneration was marked in one (No. 18) and slight in the other (No. 19). The tests were normal in a patient with marked parenchymal necrosis (No. 21) and in two with moderate parenchymal degeneration and slight focal necrosis (No. 22 and 25). The limitations of these tests in the diagnosis of some cases of portal cirrhosis is apparent.

Histochemical Studies. The histochemical observations for the liver biopsies of the seven patients with portal cirrhosis are presented in Table VIII.

No significant abnormalities of phospholipids or phosphatidic acids other than mitochondrial displacement by fat droplets were noted. Ceroid pigment was present in the livers of two patients (No. 21 and 23). Some droplets of lipids usually at least partly acidic, were present in all cases but to an appreciable degree (grade 3) in only one (No. 17). Their lobular distribution varied,

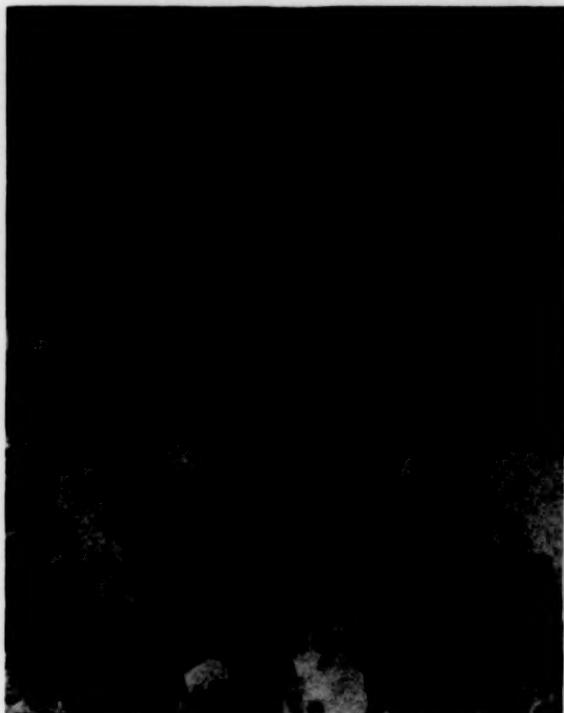


FIG. 9. Lipids: droplets of fat within the fibrous trabeculae. Frozen section (7 micra); oil red O, hematoxylin and light green; B filter, X 320.

being generalized in two cases (No. 17 and 18), centrilobular in two (No. 22 and 23), within the fibrous trabeculae in two (No. 19 and 20) and in "degenerating" cells in one (No. 21). Intratrabecular fatty cysts containing mixtures of acidic and neutral lipids were noted in two cases with marked cirrhosis (No. 19 and 20). Centrilobular fatty cysts containing neutral (No. 22) or mixed (No. 23) lipids were present in cases with moderate or mild cirrhosis, respectively.

In most instances there was no abnormality of glycogen in the liver cells. Decreased cytoplasmic glycogen in fat-filled cells was very noticeable, however, in one patient with marked cirrhosis (No. 17) and slight in the one with mild cirrhosis (No. 23). Some decrease in cytoplasmic glycogen and a 3 per cent incidence of glycogen-vacuolated nuclei were noted in the case recovering from hepatic coma (No. 21). Nuclear desoxypentose nucleic acids may have been at least slightly diminished in all but one (No. 22) of the six cases in which they were studied. Cytoplasmic pentose nucleic acids may have been decreased in two (No. 17 and 20) of five cases. Large amounts of hemosiderin were found in the parenchymal cells of two patients with marked portal cirrhosis (No. 17 and 18) and

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TABLE VI
PATIENTS WITH PORTAL CIRRHOSIS—CLINICAL DATA*

Case	Sex, Age and Color	Prior to Present Study					In Hospital		Height (in.)
		Duration ¹ of Symptoms Suggesting Cirrhosis	Known Duration of Cirrhosis	Nutrition	Diet Prescribed	Alcoholic History	Prior to Biopsy (days)	Because of	
17	F, 48 C	11 m	Diagnosis during present study	Usual wt. 136 lb.	0	Heavy beer drinker for years; some whiskey for 5 m	6	Repeated epistaxis	64 approx.
18	M, 69 W	15 m	Diagnosis during present study	4 y—205 lb.; unable to assess diet	0	Moderate wine and beer drinker	19	Inguinal hernia	68
19	M, 47 W	1 m	Diagnosis during present study	Lost from 160 lb. (max. wt.) to 130 lb. over several m; diet poor	0	At least 1 qt. wine/d for 5 y; much whiskey for 4 yr. before that	17	Hematemesis	66 approx.
20	M, 46 W	5 y	Clinical diagnosis 5 y	Diet excellent until 5 y; then poor; 5 y—220 lb. (approx); 5½ m— 203 lb.	High calorie protein, carbohydrate; moderate fat— 5½ m	Heavy drinker since youth; abstained for 2 y; then 1 pt. whiskey/d for 2 y	15	Ascites	66
21	M, 46 C	A few days	Diagnosis during present study	Until 2 y—170 lb.; then diet poor for 1½ y; 6 m—160 lb.; for 6 m diet adequate	0	Moderate drinker until 2 y; then drank heavily—½ + pt. whiskey/d and beer for 1½ y; for 6 m no alcohol	22	Hepatic coma precipitated by ?	70½
22	M, 36 W	14 m	Diagnosis during present study	Usual wt. 152 lb.; began to lose wt. 2 m; diet poor	0	Heavy drinker of whiskey, beer and wine; for 1 y ½ gal. wine/d	8	Fainting attacks, anorexia and nausea	68
23	M, 41 C	7 d	Diagnosis during present study	Usual wt. 163–169 lb.; during 1 y—157–151 lb.; diet adequate	0	Moderate drinker for years; whiskey and wine	11	Swelling of ankles and icterus	70

* Abbreviations: F—female; M—male; W—white; C—Negro; d—day; w—week; m—month; y—year; P—protein; F—fat; C—carbohydrate—in gms.; pt.—pint; gal.—gallon; Fstg.—fasting; bb—before biopsy.

† Patient received cortisone for a total of 19 days. Intramuscularly: 600 mg. for 3 days; 400 mg. for 1 day; 300 mg. for 1 day; 200 mg. for 1 day; 100 mg. for 7 days. Orally: 50 mg. for 6 days; given until the biopsy was performed. Also aureomycin, 1 gm./day for 7 days; last dose was 9 days before biopsy.

¹ Unless other information is given, "prior to present study" is to follow all abbreviated time periods noted, i. e., 11 m—11 months prior to present study.

² Height without shoes. Patients weighed wearing pajamas and robe weighing approximately 4 lb. With adjustments of height and weight, % over and under weight was calculated from tables in Duncan, G. G. Diabetes Mellitus, Principles and Treatment, pp. 268–269. Philadelphia, 1951 W. B. Saunders Co.

Ow—overweight; Uw—underweight.

³ Level of liver enlargement taken below right costal margin in midclavicular line on inspiration.

⁴ Only data considered possibly relevant noted.

⁵ Hours of fasting are recorded to the nearest half hour.

TABLE VI (Continued)

Present Study								
Weight (lb.)	Uw or Ow ² (%)	Liver ³	At Time of Biopsy		Hospital Diet Prescribed P F C	Biopsy Data		
			Other Evidence of Liver Disease	Associated ⁴ Disease		Dietary ⁵ Intake before Biopsy	Blood for Tests (A.M.)	Biopsy (A.M.)
130	14 Uw approx.	7 cm.; edge slightly round, firm, not tender; surface smooth	0	Syphilis, latent, late	Approx.: P—120 F—100 C—360	Fstg. 10 h	9:57-10:10	10:10
186	15 Ow	Liver dullness diminished	0	Gastric, ulcer, benign; syphilis, latent, late	Approx: P—45 F—60 C—175	Fstg. 9 h	8:45-8:49	8:47
131	15 Uw approx.	7 cm.; edge round, slightly tender; surface smooth	Spider angiomas; esophageal varices; splenomegaly	Syphilis, latent, late	10 d bb for 3 d approx.: P—100 F—45 C—255 For 7 d bb approx.: P—110 F—115 C—350; 500 cc. blood 2 and 1 d bb	Fstg. 10 h	9:12-9:15	9:20
148	0	3 cm.; edge round, firm, not tender; surface smooth	Spider angiomas; esophageal varices; ascites	0	For 12 d bb approx: P—105 F—100 C—350	Fstg. 9 h	9:06-9:13	9:15
142	19 Uw	Liver dullness diminished	0	0	17 d bb for 10 d approx.: P—145 F—100 C—570 For 7 d bb approx.: P—145 F—100 C—470†	Bft.: P—5 F—10 C—85 6:15-6:30 A.M.	8:35-8:55	8:45
147	5 Uw	Not enlarged	Splenomegaly; slight icterus	0	Approx: P—145 F—140 C—470	Fstg. 12 h	8:41-8:46	8:48
152	8 Uw	3 cm.; edge firm, not tender	Slight icterus	0	Approx: P—180 F—110 C—580	Fstg. 10 h	8:40-8:55	8:45

lesser amounts in those of another (No. 21) and of the patient with only moderate cirrhosis (No. 22). The pigment was noted in the Kupffer cells of only the last patient (No. 22).

Although there were some individual differences, occasionally marked ones, the histochemical findings in our seven patients with portal cirrhosis were remarkably similar: usually slight accumulations of lipid droplets, no appreciable decrease in glycogen, and perhaps some diminution in nuclear DNA. These observations may be compared with those reported by Eckhardt, Zamcheck, Sidman, Gabuzda and

Davidson⁶⁹ who studied three jaundiced patients with active alcoholic cirrhosis. They found moderate to marked amounts of fat, moderate glycogen and low cytoplasmic PNA. The larger amounts of fat found in their patients might be attributed to the greater severity of the disease or to the fact that biopsies were taken upon admission or soon thereafter, whereas none of our patients was severely ill and most had been in the hospital eleven to twenty-two days at the time of biopsy. The significance of the presence of mixed, acidic and neutral lipids in most of our cases is not known. The greater amounts of

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TABLE VII
PATIENTS WITH PORTAL CIRRHOSIS—LABORATORY DATA*

Case	Serum Bilirubin	Related to Lipid Metabolism ¹						Related to Protein Metabolism						Related to Carbohydrate Metabolism				Miscellaneous				
		Serum Cholesterol			Serum Proteins ²			CCF			Blood Sugar			Glucose Tolerance		Serum		Miscellaneous				
		Total Serum Lipids	Serum Phospholipids as Fatty Acids	Total Free Ester	Free/Total Ratio	Total Albumin/Globulin	24 hr.	48 hr.	TT	TF	SCG	SR	Normal fast-ing	80-120 mgm. %	Brom-sulfalein Retention % ³	Amylase	Lipase					
17	.63	.74	(668)	(250)	(162)	(88)	(.65)	6.0	3.92/2.08	±	1+	3.0	0	2+	2+	100	1.8	87	.6	
18	.68	1.3	(595)	(165)	(62)	(103)	(.38)	6.0	2.99/3.01	3+	4+	5.0	3+	3+	3+	90	OGT:—6 d bb; fsg: 62; 3/4 hr.—191; 1 hr.—234; 2 hr.—203; 3 hr.—168	1.4	21.5	101	.5
19	1.4	2.5	(505)	(174)	(64)	(110)	(.37)	7.2	3.59/3.61	3+	4+	3.0	2+	0	0	108	OGT:—5 d bb; fsg: 123; 3/4 hr.—183; 1 hr.—193; 2 hr.—167; 3 hr.—193	3.0	40.1	130	1.6
20	.37	.81	(705)	(176)	(76)	(100)	(.43)	6.16	3.65/2.51	2+	2+	1.0	0	0	0	119	1.3	12.8*	100	.5
21	2.4	3.1	(785)	12.0	11.9	203	54	149	.27	3.98/3.86	0	0	1.5	0	0	0	224	OGT:—8 d pb (6 d after last cortisone); fsg: 112; 3/4 hr.—234; 1 hr.—264; 2 hr.—247; 3 hr.—166	5.2	12.0*	282	2.6

22	1.3	2.4	(1185)	14.7	24.7	448	174	274	.39	6.88	0	0	1.0	0	0	0	0	104	9.8	14.5*	68	.2
										4.18/2.70								94; $\frac{1}{2}$ hr—180; 1 hr—156; 2 hr—144; 3 hr—127; 4 hr—76; 5 hr—86; 6 hr—92				

* Abbreviations: ... tests not performed; d—day; bb—before biopsy; pb—after biopsy; hr.—hour; CCF—cephalin cholesterol flocculation; TT—thymol turbidity; SCG—serum colloidal gold; SR—scarlet red; OGT—oral glucose tolerance.

¹ Determinations indicated by parentheses in tests "Related to Lipid Metabolism" were performed at the Graduate Hospital. Those without parentheses were performed in the laboratory of Dr. I. J. Pincus, Jefferson Medical College.

² The results of serum protein studies performed by paper electrophoresis and by the Tiselius method in some of these patients, are described in the text.

³ In all cases but those indicated by *, the bromsulfalein test was performed within three days before or after the biopsy. In the remaining cases this test was performed as follows: Case No. 17—5 d bb;

20—14 d bb; 21—19 d pb; 22—4 d pb.

glycogen in the liver cells of our patients may be ascribed to the diet which they had received. Eckhardt and his associates noted increases in liver glycogen after feeding high carbohydrate or generally nutritious diets. In their three subjects the cytoplasmic PNA was initially low but gradually increased when a nutritious diet was fed. Perhaps a similar change had occurred in our patients in which the cytoplasmic PNA was undiminished (No. 18, 21 and 23). However, two patients (No. 17 and 20) who were in the hospital for similar periods and had received equally good diets still showed diminished PNA indicating, perhaps, that other factors are involved. It would appear that the three histochemical changes generally noted in our patients with portal cirrhosis may be due to the inadequate dietary intake which is so frequent among such patients, especially those consuming large amounts of alcohol. The changes are not characteristic of portal cirrhosis alone. The possible role of a deficiency of lipotropic factors⁶² is suggested by the centrilobular distribution of the fat droplets in two cases (No. 22 and 23).

Ceroid, a lipid pigment which is insoluble in organic solvents, was first described as occurring in the cirrhotic livers of choline-deficient rats⁶³ but has since been found elsewhere. Its presence in the liver of the patient recovering from hepatic coma (No. 21) is noteworthy. It may be regarded as evidence of previously more extensive fatty changes and hepatocellular necrosis. Ceroid has also been found in the livers of dogs recovering from experimentally induced hepatic coma.⁶⁴

The significance of the occurrence of appreciable amounts of hemosiderin in the parenchymal cells of only those patients in whom there were no fatty cysts is not clear. It may favor a different etiology for the portal cirrhosis in the two groups of cases.

Ten patients with various hepatic diseases were studied in the same manner as those with diabetes mellitus or portal cirrhosis. There was one patient with primary biliary cirrhosis. One had secondary biliary cirrhosis due to carcinoma of the ampulla of Vater, and one patient had portal cirrhosis and secondary biliary cirrhosis caused by carcinoma of the pancreas. Primary carcinoma, secondary carcinoma or Hodgkin's disease involved the livers of three patients. Two with sickle cell anemia and two patients recovering from viral hepatitis completed this "miscellaneous" group.

The laboratory findings in all of these pa-

tients were in keeping with the disease processes present. Striking elevations of the total serum lipids and all fractions were noted in the patients with biliary cirrhosis.

The outstanding feature of the histochemical findings in these ten patients with various types of liver disease was that the changes were

each of the many factors possibly playing a role in the accumulation of cytoplasmic lipids can be little more than speculative.

COMMENTS

Throughout the investigations reported here, carefully correlated clinical, laboratory and

TABLE VIII
PORTAL CIRRHOSIS—HISTOLOGIC AND HISTOCHEMICAL OBSERVATIONS

No.	Histologic Observations ¹				Histochemical Observations ²							
					Lipids ³		Glycogen ⁴		Nucleic Acids ⁵		Iron Pigment	
	Lobul. Destr.	Necrosis	Paren. Degen.	Conn. Tis.	Cytoplasmic Droplets	Cysts	Cytoplasmic	Nuclear	Nucl. DNA	Cyto. PNA	Parenchymal Cells	Kupffer Cells
17	Mkd.	0	Areas, occ. mkd., near c.t.	Mkd.	2 med. and lg., neut. and fine, ac., genl.	0	4 in cells with little or no fat 0-1 in fat-filled cells	0	2	2	3 fat-free 1 fat-filled cells	0
18	Mkd.	0	Mkd., diffuse	Mkd.	1 med., ac., genl.	0	4 unif.	0	2	3	3	0
19	Mkd.	0	Sl. diffuse	Mkd.	tr. med. ac. and neut.*	1 sm., ac. and neut., trabec	4 unif.	0	0	0
20	Mkd.	0	Sl. diffuse	Mkd.	tr. med. ac. and neut.*	1 sm., ac. and neut., trabec	4 unif.	0	1	1	0	0
21	Mkd.	Mkd. focal	Mkd. diffuse	Mkd.	f. tr. fine neut., in "degenerating" cells	0	4 unif.	0	1½	4	1	0
22	Mod.	Mod. focal	Mod. diffuse	Mod.	1 lg., neut. centr.	3 lg., neut., centr.	3 unif.	3	4	..	tr.	tr.
23	Sl.	Sl. focal	Mod. diffuse	Sl.	1 med. and lg., ac. and neut., centr.	2 med. and lg., ac. and neut., centr.	4 sl. decr. in cells containing fat	0	2	4	0	0

¹ Histologic observations:

Lobul. destr.—Destruction of lobular architecture

Paren. degen.—Parenchymal cell degeneration

Conn. tis.—Connective tissue as demonstrated by Mallory stain

Grading—0—not present; sl.—slight; mod.—moderate; mkd.—marked.

² Grading for observations on lipids, glycogen, nucleic acids and iron pigment, ranges from 0 to 4 and includes within the interval 0-1, v.f.tr. very faint trace), f.tr. (faint trace), and tr. (trace).

³ Lipids: Separate data are given for cytoplasmic fat droplets and for fatty cysts as follows:

Size: v.sm.—very small; sm.—small; med.—medium; lg.—large.

Reaction: ac.—acidic; neut.—neutral; cryst.—crystals also present.

Distribution within hepatic lobule: genl.—general; port.—periportal; cent.—central; scat.—scattered; trab.—intratrabecular; *—present in the few cells enmeshed in the bands of connective tissue.

⁴ Glycogen:

Cytoplasmic—Distribution: unif.—uniform; varn.—variation; mkd.—marked; mod.—moderate; decr. port.—decreased in periportal regions; sl. decr.—slight decrease.

Nuclear—percentage of nuclei containing some glycogen.

⁵ Nucl. DNA—Nuclear desoxyribose nucleic acid.

Cyto. PNA—Cytoplasmic pentose nucleic acid.

qualitatively similar although individual quantitative variations were observed. Thus, usually some accumulation of lipid droplets, either acidic or neutral, slight to moderate reductions in cytoplasmic glycogen, a possible diminution of nuclear DNA and, occasionally, reduction of cytoplasmic PNA were noted. Assessment of

microscopic studies have been carried out in patients with diabetes mellitus, portal cirrhosis and other hepatic diseases. Biochemical examinations of blood were made at the same time that histologic and histochemical examination of liver tissue was performed to permit precise correlation of all findings. The results have not

only contributed to the understanding of certain diseases affecting the liver but have posed new problems and re-emphasized old ones.

Classic concepts of the pathology of liver diseases have been generally based on the terminal stages as studied after death. Rarely have minor hepatic abnormalities in patients not dying of a liver disease been correlated with any signs or symptoms that may have been present during life.

The newer methods of study employed during this investigation should help to answer certain basic questions. From the histologic viewpoint, what constitutes a normal liver and what is the significance of minimal morphologic abnormalities? Likewise, histochemically, what are the findings in a normal liver and what significance should be attached to slight deviations from these?

Liver biopsy obviates the agonal and *post-mortem* changes encountered in autopsy studies. The findings in tissue thus obtained from living persons should afford valuable information regarding histologic and physiologic changes heretofore not understood. Their interpretation, however, requires a new concept. If a patient has clinical or biochemical evidence of hepatic dysfunction, even a slight deviation from the apparent normal may assume importance. Existing methods of investigation have revealed no correlation between slight histologic changes and clinical and laboratory configurations. It would appear that in some hepatic diseases extensive morphologic changes may occur without demonstrable functional disturbances, and that, conversely, marked abnormalities of function may be associated with minimal or no changes in hepatocellular structure. The functional and structural changes occurring during any hepatic disorder are determined not only by the nature and the duration of the affection but also by the balance achieved between destructive and regenerative processes. While liver function tests reflect biochemical "lesions" and histologic methods reveal morphologic ones, histochemistry attempts to bridge the gap between the two types of investigation. Nevertheless, not all of the diversified activities of the liver can yet be assessed by utilization of functional, histologic and histochemical methods combined. Many so-called liver function tests are non-specific. Even though histochemistry aims to localize the components of physiologic processes within cells and tissues and to correlate changes

in them with alterations in functional states, at present it often reveals only the end results rather than the initial and intermediate steps in such dynamic processes. Despite these limitations, the information provided by these combined methods does contribute to our understanding of the mechanisms underlying various diseases. It should be emphasized, however, that alterations in structure and function may not always occur simultaneously or at the same rate. Observations made at only one time may therefore fail to reveal relationships which become evident only in serial investigations. Eckhardt and his associates,⁵⁹ for example, have noted that improvement in function may precede evidence of histologic improvement and that clinical condition and function need not parallel histochemical findings.

The appearance of lipids demonstrable by available histochemical methods in cells in which they are not normally found may be taken as evidence of altered cellular metabolism. Initially, this may be the result merely of a change in the physicochemical state of intracellular lipids rather than an actual increase in them as shown by chemical analysis. Further accumulation of demonstrable lipids, however, is approximately paralleled by a rise in the chemically determined fat content. The amount of fat depends to some extent upon the relative availability of the various nutrients, for example, during fasting or when unusually large amounts of fat are ingested, lipids accumulate in the liver, representing the previously normal organ's response to abnormal situations. Under other circumstances, however, an apparently similar type of fatty metamorphosis may denote abnormal function due to some cause such as a specific dietary deficiency, some metabolic disease or hepatotoxic condition. One or more of the following processes may be concerned: (1) excessive mobilization of fat to the liver, (2) impaired hepatocellular lipid metabolism or (3) decreased fat transport from the liver. As an intracellular process, fat accumulation presents a number of problems closely related to those of lipid metabolism in general. Although the chemical transformations effected by the hepatic cells are moderately well understood, relatively little is known regarding the exact intracellular sites where these take place. Objective assessment of the fatty changes which may be found in human liver biopsies are further hampered by a lack of certain fundamental facts such as the

minimum times required for the appearance and disappearance of these changes and the types of lipids involved.

Histochemically demonstrable lipids were found in the livers of fourteen of the sixteen diabetic patients. In only seven were their livers clinically enlarged, but in this small group there

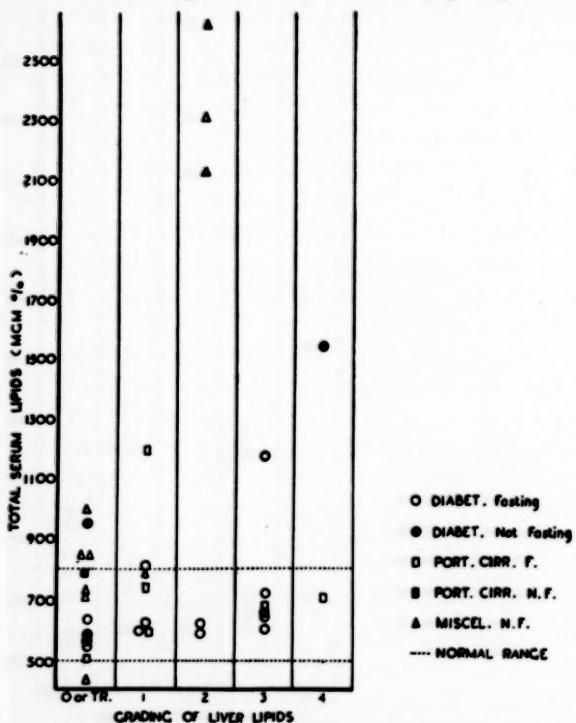


FIG. 10. Chart demonstrating the lack of correlation between the blood lipid concentrations and the grades for histochemically demonstrable lipids in the livers of thirty-three patients in whom blood and liver samples were taken simultaneously.

appeared to be a tendency for hepatomegaly to be associated with greater degrees of fatty change. Further analysis of these cases indicated that such enlargement in adult diabetics may suggest the co-existence of hepatic disease not necessarily related to their diabetes. Interesting, but possibly not significant, was the greater degree of fatty change usually noted in female diabetics which may have been related to the duration of their disease but not to its "severity" or to their percentage overweight. Diabetes was known to have existed in seven of the sixteen patients for five weeks or less. Of these seven patients four were women. The degree of fatty metamorphosis was greater in this group recently diagnosed than in those whose disease was of longer known duration. No correlation between age and degree of fatty metamorphosis was noted.

In neither the diabetics nor the cirrhotics was there any correlation between the amounts of histochemically demonstrable liver lipids and the percentages over- or underweight. In the entire group of thirty-three patients no correlation was found between the degree of fatty change in the liver and abnormalities in any of the liver function tests. Ulevitch, Gall, Abernathy and Schiff⁶⁶ likewise found no parallelism between the results of such tests and fatty metamorphosis.

No reports could be found in the literature concerning the relation of blood lipids to histochemically demonstrable liver lipids determined simultaneously. Zimmerman and his co-workers⁴⁶ compared blood cholesterol concentrations with the amount of lipids seen in sections of liver but they do not specify the exact conditions under which their studies were performed. Man and his associates⁶⁶ evidently found no relationship between serum lipids determined while the patient was in the postabsorptive state some time before death and the liver lipids estimated chemically at autopsy. Since certain of our observations suggest that changes in histochemically demonstrable lipids may occur as rapidly in humans as in experimental animals, it is essential that all pertinent conditions at the time of investigation be clearly specified. The simultaneous aspiration of blood and liver tissue in this study permitted close comparison of the corresponding constituents. As is shown in Figure 10, there was no correlation between the level of the serum lipids and the quantities of liver lipids demonstrable histochemically. Extreme examples are provided by the patients with primary or secondary biliary cirrhosis in whom the serum lipids were greatly elevated while only small amounts of liver lipids were demonstrable. The absence of chylomicronemia and the lack of droplets of free lipids in the cytoplasm of the hepatic cells may be related in these cases. Similar studies (to be published by Dr. I. J. Pincus⁶⁷) on dogs and rabbits under various conditions have also revealed a lack of correlation between the serum and chemically determined liver lipids. On the other hand, Kaplan and Chaikoff⁶⁸ found a decrease in blood lipids to be associated with elevation of liver lipids in pancreatectomized dogs while Beveridge and Johnson⁶⁹ reported that increases in blood fatty acid and total cholesterol levels accompanied corresponding increases in the liver lipid fractions in alloxan diabetic rats. Additional knowl-

edge regarding the metabolic interrelations of depot, blood and liver lipids may elucidate the significance of these divergent findings.

Among the thirty-three patients included in this study, there was no definite relationship between the quantity or reaction of demonstrable liver lipids and the amounts of cytoplasmic or intranuclear glycogen. That there is not necessarily any decrease in liver glycogen in the presence of increased liver lipids has been reported by Warren and LeCompte.⁶⁵ In our patients no correlation was found between the hepatic lipids and the blood glucose values at the time of biopsy or the mean blood sugar levels prior to biopsy. There was no constant relationship between the amounts of liver lipids and the desoxypentose or pentose nucleic acids.

The blood glucose levels at the time of biopsy are compared with the amounts of liver glycogen for the thirty-three patients in Figure 11. No relationship was found between the two or between the liver glycogen and the mean of several fasting blood sugar levels determined prior to biopsy. Hildes and his associates⁷⁰ also found no correlation between liver glycogen as estimated biochemically or histochemically and the fasting blood sugar level, the type of diabetes or the degree of ketosis. In our cases liver glycogen was unrelated to the amounts of the nucleic acids or the iron pigments in the parenchymal or Kupffer cells.

Among the important functions of the liver are the synthesis and storage of glycogen. This polysaccharide is abundant in the cytoplasm of the liver cells after the ingestion of carbohydrates. During fasting or starvation it decreases and may even disappear. Normally, however, glycogen is not found in the nuclei of the hepatic cells at any time. Thus its deposition within the nuclei, giving them a vacuolated or "ballooned" appearance, is abnormal. This occurred to some degree in thirteen of sixteen diabetics, in one of the seven patients with portal cirrhosis and in six of the ten with other liver diseases. Its possible significance and its reciprocal relation to the amount of cytoplasmic glycogen was considered in the section on the diabetic patients. No parallelism between the incidence of this change in the twenty patients in which it was observed and their blood sugar levels at the time of biopsy or the mean of those determined before the biopsy could be established. This is in agreement with the studies reported by Chipps and Duff.⁶⁶ Zimmerman and his co-

workers,⁴⁸ on the other hand, believed that there might be some relation between nuclear vacuolation and blood sugar levels. We could establish no correlation between the degree of glycogen vacuolation and the state of control of their disease or the administration of insulin in the diabetics.

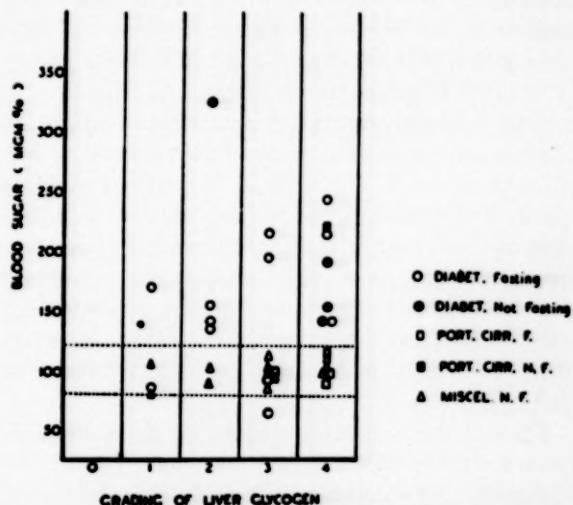


FIG. 11. Chart demonstrating the lack of correlation between the blood sugar concentrations and the grades for histochemically demonstrable glycogen in the livers of thirty-three patients in whom blood and liver samples were taken simultaneously.

No significant correlation could be established between the amounts of nuclear DNA and any other hepatocellular constituents or other observations on our patients. The total serum proteins and their fractions were unrelated to the cytoplasmic PNA values. In one patient (No. 5), however, elevated serum proteins (9.4 gm. per cent) were associated with the greatest amount of cytoplasmic PNA observed. In some instances decreased PNA was found in patients with histories of recent undernutrition.

SUMMARY

In thirty-three patients the clinical and biochemical findings were correlated with histologic and histochemical observations on liver tissue obtained by needle biopsy. There were sixteen patients with diabetes mellitus, seven with portal cirrhosis but no diabetes and ten with other liver diseases. The simultaneous study of blood and liver tissue permitted a precise comparison of the corresponding constituents.

Among some of the diabetic patients abnormalities of serum proteins and bromsulfalein retention were noted frequently, and abnormali-

ties in the turbidity and flocculation tests infrequently. Focal lesions, considered to be of necrotic origin, and degenerative changes in the parenchymal cells occurred in sixteen of nineteen diabetic patients. Similar lesions were also found in the livers of three of the seven patients with portal cirrhosis and six of the ten with other liver diseases. Therefore they are not characteristic of the diabetic state. It is suggested that if these changes are diffuse they may be related to impairment of hepatic function.

Our findings suggest that changes in hepatic lipids may be as dynamic in humans as they are in experimental animals. It is strongly recommended that when liver biopsies are taken for the purpose of assessing histochemically demonstrable changes, the exact conditions existing at the time should be clearly specified, including such details as the state of nutrition, obesity, diet, duration of fasting and any form of therapy.

During the accumulation of lipids in the diabetic liver the neutral fats are most extensively involved. No correlation could be established between the degree of fatty metamorphosis of the liver and any other histochemical or laboratory observations. Among the diabetic patients there was a suggestive correlation between marked increases of lipids and hepatomegaly. The accumulation of lipids within the parenchymal cells was not found to antagonize the deposition of cytoplasmic glycogen to any great extent. Intracellular glycogen, giving rise to vacuolation or "ballooning," occurred to some degree in thirteen of the sixteen diabetic patients, in one of the seven with portal cirrhosis and in four of those with various other liver diseases. The reciprocal relationship between the amounts of nuclear and cytoplasmic glycogen reported to occur in diabetic patients was confirmed. For these studies the periodic acid-Schiff's reaction, controlled by amylase hydrolysis, was employed to verify previous reports based on Best's carmine method for glycogen.

Only possibly moderate variations in nuclear desoxypentose nucleic acids were noted among the different patients. However, the DNA content of the nuclei of the parenchymal cells in any given patient was relatively constant. A similar constancy was noted for cytoplasmic pentose nucleic acids but there was a greater difference between patients. In some instances, apparently marked diminutions could be correlated with previous undernutrition. Iron was found in the

parenchymal and Kupffer cells of eight of the sixteen diabetic patients. It could not be correlated with the accumulation of lipids or with previous blood transfusions.

No correlations could be established between the parenchymal and mesenchymal histologic findings and abnormalities in liver function tests in the simultaneous studies on the seven patients with portal cirrhosis. The significance of intratrabecular fatty cysts in determining whether portal cirrhosis has been preceded by a fatty liver would not appear to be as great in needle biopsy material as in autopsy studies.

No relationship was found to exist between the amounts of histochemically demonstrable liver lipids and the blood lipid levels or between the liver glycogen and blood sugar concentrations.

In some instances uneven, regional variations in the distribution of histochemically demonstrable liver lipids and glycogen were found in different portions of the same specimens obtained by needle biopsy.

Acknowledgments: The authors gratefully acknowledge the valuable assistance given by Dr. I. J. Pincus and the encouragement and advice of Professor C. H. Best. Professors H. J. Barrie and W. S. Hartroft and Dr. A. M. Valdes-Dapena were consulted regarding various problems in histopathology. Drs. H. J. Tumen, J. T. Beardwood, Jr., T. A. Johnson, J. H. Willard, L. H. Beizer, J. W. Hundley, C. R. Tittle and J. L. A. Roth kindly permitted study of patients under their care. Initially, the liver biopsies were taken by Dr. V. Bressler. We are grateful to Dr. G. R. Williams who performed the paper electrophoretic studies and to Dr. D. W. Clarke for the electrophoretic analyses. The histochemical studies were made with the technical assistance of Mr. O. T. George and were partly supported by a grant from the National Research Council, Canada. Thanks are due to Mrs. M. Travis, Mrs. J. Krautheim, Mrs. K. Salter and to many others who helped in various ways during these investigations.

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Studies on Myocardial Metabolism*

IV. Myocardial Metabolism in Diabetes

I. UNGAR, M.D., M. GILBERT, M.D., A. SIEGEL, M.S., J. M. BLAIN, M.D. and R. J. BING, M.D.

Birmingham, Alabama

STUDIES on metabolism in diabetes have been mainly concerned with changes in the metabolism of the organism as a whole, or of the liver specifically.¹⁻³ Few studies have been carried out on the diabetic heart *in situ*.

It is likely that more than one metabolic disturbance is present in diabetes. Difficulties in transfer of sugar across the cell membrane, interference with the hexokinase reaction, a defect in oxidative phosphorylation connected with the generation of high energy phosphate, and disturbances in synthesis of fatty acids have all been implicated.¹ A study of the metabolism of individual foodstuffs, using glucose labeled with C-14, has indicated that a marked decrease in the rate of total oxidation of sugar occurs in rats made diabetic with alloxan.³ Similar results were obtained by Feller and his associates in pancreatectomized dogs.⁴ It is likely, however, that this reduction in the rate of total oxidation of glucose in the diabetic animal is only relative because, although utilization of glucose is less than in normal animals with similar hyperglycemic levels, it is still above that of the normal animal at its usual normal blood sugar level.⁵

Glucose seems also to be utilized by the diabetic heart in the heart-lung preparation. Patterson and Starling⁶ found that the diabetic heart *in vitro* has not lost its ability to consume sugar. The diabetic dog's heart in the heart-oxygenator system also uses sugar, although to a diminished degree.⁷ Cruickshank⁸ believed that the isolated heart progressively loses its power to burn sugar when the preparation is made from animals from two to nine days after total pancreatectomy. Using the intact heart of depancreatized dogs *in situ*, Himwich⁹ found a positive myocardial glucose balance; the ability of cardiac tissue to glycolyze was retained in pancreatic diabetes.

* From the Department of Medicine and Physiology, University of Alabama Medical Center, Birmingham, Ala. Work supported by the U. S. Public Health Service Grant No. H-1129(CS), The Life Insurance Medical Research Fund and the American Heart Association.

Other disturbances in carbohydrate metabolism are probably consequent to those of glucose. Cardiac tissue slices from diabetic dogs utilize pyruvate and lactate less readily than those from normal animals.¹⁰ Similarly, diminished utilization of C-14-labeled pyruvate has been observed in both cardiac and diaphragmatic muscle of diabetic animals and the proportion of pyruvate converted to CO₂ was decreased.¹¹ The diabetic heart in the heart-lung preparation, however, showed no deficiency in lactate utilization.⁷ Using pyruvate labeled with C-14, Hastings¹¹ found that the addition of insulin *in vitro* increased the conversion of labeled pyruvate to C-14 CO₂ in the diaphragm of the diabetic animal but not in cardiac muscle. Osborn¹² made the observation that insulin reduced the conversion of pyruvate labeled with C-14 CO₂, thus shifting the metabolism of the C-2 intermediates derived from pyruvate from an oxidative phase to one involving synthesis. Bueding and associates¹³ observed a marked rise in blood pyruvate and lactate following insulin injection into depancreatized dogs. This was ascribed to increased production of these metabolites by the tissues. It is possible that there is some connection between these results and those of Foa¹⁴ who showed that in the liver of alloxan-diabetic rats the percentage of thiamine phosphorylated to cocarboxylase was significantly less than in the normal. The cocarboxylase content of liver tissue increased when the diabetic animals were treated with thiamine and insulin.¹⁵

The disturbances in fatty acid metabolism are probably related to those in carbohydrate metabolism, since it is likely that the transfer of energy required for fat synthesis is derived mainly from coupled reactions involving the simultaneous oxidation of some normal carbohydrate intermediate.¹⁶ Thus the diabetic state is

accompanied by a marked decrease in the ability to synthesize fatty acids from glucose, lactate or pyruvate.^{12,17} According to Brady,¹⁶ insulin does not reverse this defect. The accumulation of fat in diabetic organs is probably the result of increased mobilization from fat depots and not of increased synthesis of fat in those organs. According to Cruickshank,¹⁸ the diabetic heart in the heart-lung preparation primarily utilizes protein and/or fat as sources of energy. It has been shown that the normal human heart utilizes fatty acids.¹⁹ Myocardial extraction of fatty acids was particularly great after a high fat intake, suggesting storage of fat in the heart muscle.¹⁹

Amino acid metabolism in diabetes has been studied by Luetscher²⁰ who found high fasting plasma amino acid levels in patients with severe untreated diabetes. This may be the result of deficiencies in protein synthesis, which appears to be dependent on glucose utilization.^{1,21}

It was the purpose of the study reported in this paper to investigate the myocardial metabolism of carbohydrates, fatty acids, amino acids and ketone bodies of patients with diabetes mellitus and of dogs made diabetic with alloxan, using the method of coronary sinus intubation.^{22,23} The findings will be compared with those obtained on the heart of non-diabetic patients and animals.

MATERIALS AND METHODS

Patients. Twenty-two patients without known metabolic abnormalities were selected as controls. The data on sixteen of these were published in previous papers of this series.^{19,24} The remainder were studied during this investigation. Table I summarizes the findings in these patients.

Seven diabetic patients from the Diabetic Clinic were investigated. (Table I.) All but one were female. They varied in age from twenty-one to fifty-seven years and the duration of diabetes ranged from five months to eighteen years. Insulin was required in all the diabetic patients except one. This patient was maintained on diet alone. The insulin requirements of the remainder varied from 30 to 75 units daily. The diabetes was reasonably well controlled in all patients. Insulin was withheld from five individuals seventy-two hours prior to the experiments. In two patients it was withheld twenty-four hours before the investigation. (E. C. and B. R., Table I.)

The patients of both the control and diabetic series were fasted twelve hours prior to the

studies. The coronary sinus of these patients was intubated.^{22,23} Arterial blood was obtained from the femoral artery. Simultaneous blood samples were drawn from the coronary sinus and femoral artery for analyses. Coronary blood flow was determined by the method of nitrous oxide desaturation.^{23,25} Mixed venous blood samples were obtained from the right ventricle or pulmonary artery and the cardiac output was determined by the Fick principle. The patients were not subjected to anesthesia or sedation.

Dogs. Nine mongrel dogs were used in this study. (Table II.) Good nutrition was assured by an adequate diet,* particularly in the three to five days prior to the experiment. The animals were anesthetized with 4 per cent nembutal[†] given intravenously. The level of anesthesia was maintained at a steady plane throughout the experiment, as manifested by respiratory rate (16–20/min.), mean arterial blood pressure (120 to 140/mm. Hg) and pulse rate (120 to 160/min.).

Air samples were collected and nitrous oxide administered via a Blalock mask applied over the mouth and nose of the dogs.²⁶ Adequate aeration was ascertained at all times.

The external jugular vein was isolated and intubated. With the dog lying on the right side, the catheter was inserted into the coronary sinus under fluoroscopic control. A femoral artery was isolated and intubated. Simultaneous blood samples were drawn for metabolic studies and cardiac output was obtained.²⁷

Several days following control studies the dogs were given alloxan, 0.75 mg./kilo intravenously, in a 2 to 5 per cent normal saline solution. Five to eight days after the diabetic state was established, as manifested by sugar in the urine and a positive oral glucose tolerance test,† the procedure followed in the control experiment was repeated. Regular insulin‡ (40 units) was given intravenously. One and a half hours later, simultaneous coronary sinus and femoral artery blood samples were again drawn for metabolic analy-

* The dog diet consisted of Gold Seal Horse Meat, Gold Seal Products, 1 pound per day and Jim Dandy Ration Pellets, Western Grain Co. The content of the latter is as follows: crude protein 24 per cent; crude fat 6.0 per cent; crude fiber 5 per cent and nitrogen-free extract 45 per cent.

† The stomach was intubated by means of a Levin tube passed through the mouth and 50 per cent glucose solution was given, 2 gm./kilo. Venous blood samples were drawn two and three hours later, respectively, and analyzed for sugar.

‡ Eli Lilly and Company, Indianapolis, Inc.

ses, and the coronary blood flow determination was repeated.

Procaine penicillin was given prophylactically and for several days after each phase of the experiment. To minimize the effects of anemia resulting from large volume of blood drawn for analyses, each dog received 200 cc. of dog whole blood which was given at the conclusion of the control experiment. The dogs were fasted twelve to sixteen hours prior to each test.

Blood glucose was determined by the method of Hagedorn and Jensen²⁸ using Somogyi's method to prepare the blood filtrates.²⁹ Pyruvate was determined according to the method of Friedemann and Haugen³⁰ using a trichloroacetic acid filtrate. Lactate was measured by the method of Barker and Summerson.³¹ The manometric method of Van Slyke and Neill³² was used for the blood oxygen analysis. Fatty acids were determined according to the method of Man and Gildea.³³ This is essentially a modification of the procedure of Stoddard and Drury's volumetric analysis.³⁴ In our laboratory the maximum error of this method is 1.5 per cent. Amino acids were determined according to the method of Albanese and Irby.³⁵ Ketones were determined by a modification of the micro-method of Greenberg and Lester.³⁶ The extractions by the myocardium of oxygen and foodstuffs were calculated by obtaining the difference between their respective arterial and coronary sinus blood values. The myocardial usage of these substances per 100 gm. left ventricle per minute equalled their respective extraction multiplied by the coronary flow and divided by 100. The oxygen extraction ratio percentages equalled their respective oxygen equivalent (glucose [0.75], pyruvate [0.64], lactate [0.75], amino acids [4.68], fatty acids [570], ketones [1.02]) times their extraction by the myocardium, times 100 and divided by the myocardial oxygen extraction of the metabolite in question. The contribution of each metabolite to the energy requirements of the heart is shown by these ratios if complete catabolism is assumed. In calculating the oxygen extraction ratios of various metabolites complete oxidation of food-stuffs to CO_2 and H_2O is assumed. This does not occur in every instance because of storage and conversion within the metabolic pool. However, it is believed that the use of the ratio is justified since it affords some basis for comparison. For example, if the ratios are very large for any particular metabolite, far exceeding oxidative

requirement, storage or conversion is suggested. On the other hand, if the ratios are small, other substances must have been used to a greater degree to satisfy the oxidative requirements of the heart.

All data were subjected to statistical analysis.³⁷ Probability was obtained from R. A. Fisher's³⁸ tables by finding the value corresponding to the "t" and degrees of freedom of the entrees.

RESULTS

Results in Patients. Table I shows that the mean values for myocardial usage of all carbohydrates were diminished and those of non-carbohydrates increased. The diminution in myocardial extraction and usage of lactate and the increase in myocardial extractions and usage of fatty acids were statistically significant. (Table III.) The myocardial oxygen extraction ratio of carbohydrate was decreased and that of non-carbohydrates increased. (Table I.) Although the non-diabetic human heart already derives the major portion of its fuel for energy production from non-carbohydrate material, the proportion of non-carbohydrate to carbohydrate foodstuffs used by the diabetic heart is much greater (55 to 70 in the normal as compared to 23 to 309 in the diabetic). Apparently the diabetic human heart, although able to utilize glucose, is relatively deficient in its utilization since at equally high arterial glucose concentration the expected myocardial usage of this foodstuff should be significantly greater.³⁴ The finding of diminished myocardial lactate consumption is in contrast to the results obtained by Evans⁷ with the heart-lung preparation. Shorr,¹⁰ on the other hand, found that cardiac tissue from diabetic dogs used lactate and pyruvate less readily than that from normal animals. Increased myocardial fatty acid usage had previously been noticed in the diabetic heart *in vitro*.³⁹ It is noteworthy that the increased usage of fatty acids occurred in the absence of elevated arterial concentration of fatty acids. (Table I.)

No significant changes in arterial levels of lactate, amino acids and ketones were noted. (Tables I and III.) An elevation of blood concentration of ketones in severe uncontrolled diabetes is well known. A rise in concentration of amino acids in patients with severe diabetes mellitus has also been described, probably a result of deficiency in protein synthesis.^{1,20} Normal blood levels of these substances in the patients of this

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TABLE I
RESULTS IN NON-DIABETIC AND DIABETIC HUMAN SUBJECTS

Patient	Car- diac Index	Left Ven- tricular Work (kg./min.)	Coronary Flow	Arterial O ₂ Vol. % Δ	Blood Levels and Myocardial Extractions						Ketones (mg./100 cc.)						
					Glucose (mg. %)		Pyruvate (mg. %)		Lactate (mg. %)		Amino Acids (mg. N/100 cc.)						
					Arterial	Δ	Arterial	Δ	Arterial	Δ	Arterial	Δ					
<i>Non-diabetics</i>																	
<i>Diabetics</i>																	
E. C.	10.4	26.6	119.5	12.0	82.6	3.4	0.452	0.087	5.14	0.84	2.88	0.03	1.022				
B. R.*	3.14	7.81	125.0	7.5	78.5	1.8	0.398	0.028	5.0	1.02	3.94	0.2	1.922				
M. D.	3.75	9.48	86	11.22	254.4	0.06	0.389	0.074	5.63	1.23	4.45	0.14	0.970				
A. M. M.	3.63	7.45	104	12.0	265	5.7	0.386	0.147	6.88	0.73	3.8	0.31	1.62				
A. M.	2.60	5.8	109	12.7	202	2.0	0.405	0.057	7.0	0.72	3.4	0	1.385				
R. J.	2.4	4.5	64.7	11.04	204	2.0	0.436	0.094	6.9	0.3	3.42	0.01	1.075				
M. E.	4.59	89.0	8.55	252	2.4	1.710	0.220	6.46	0.24	3.85	0.23	1.438	0.08				
Mean	99.6	10.71	191.21	2.48	0.38	0.1	6.14	1.03	3.68	0.83	1.347	0.071				
Range	64.7-125.0	7.5-12.7	78.5-265.0	0.06-5.70	0.171-4.52	0.03-0.22	5.0-7.0	0.30-2.40	2.88-4.45	0.3-10	0.97-1.922	0.02-0.19				
<i>O₂ Extraction Ratio (per cent)</i>													O ₂ Extraction Ratio (per cent)				
<i>Myocardial Usage (100 gm. left ventricle per minute)</i>																	
Patient	O ₂ Vol. %	Glucose (mg.)	Pyruvate (mg.)	Lactate (mg.)	Amino Acids (mg. N)	Ketone (mg.)	Fatty Acids (mEq.)	Glucose	Pyruvate	Lactate	Amino Acids	Fatty Acids	Ketones				
<i>Non-diabetics</i>													Total Non- carbo- hydrate				
E. C.	14.4	4.1	0.104	1.01	0.035	0.12	2.02	21.3	0.465	5.23	1.21	468.0	14.2				
B. R.*	9.4	2.3	0.035	1.28	0.25	0.108	1.03	18.0	0.240	10.2	13.0	600.0	11.0				
M. D.	9.6	0.51	0.064	1.06	0.12	0.026	0.86	4.03	0.423	8.22	6.04	138	8.8				
A. M. M.	13.8	5.9	0.153	0.76	0.322	0.021	0.062	35.5	0.78	4.26	12.6	86.6	5.0				
A. M.	13.9	2.18	0.062	0.788	0	0.065	1.190	11.8	0.288	4.25	0	245	8.6				
R. J.	7.24	1.29	0.060	0.195	0.004	0.020	0.31	13.6	0.548	2.04	0.42	140	4.3				
M. E.	7.6	2.14	0.190	0.210	0.20	0.071	1.26	21.2	1.65	2.1	12.5	376	16.6				
Mean	10.84	2.63	0.081	0.76	0.13	0.062	0.962	17.92	0.628	5.18	6.54	293.4	9.8				
Range	7.26-14.4	0.51-5.9	0.035-1.53	0.2-1.28	0.0-3.22	0.02-0.12	0.06-2.02	4.03-35.5	0.24-1.65	2.04-10.2	0-13.0	86.6-600	4.3-16.6				
													12.67-40.77				
													104.2-624				

* Also Kinnier-Wilson disease.

TABLE II
CONTROL AND ALLOXAN-DIABETIC DOGS; DIABETIC DOGS GIVEN INSULIN

Dog. No.	Glucose (mg. %)		Pyruvate (mg. %)		Lactate (mg. %)		Amino Acids (mg. N/100 cc.)		Fatty Acids (mEq./100 cc.)		Ketones (mg./100 cc.)		On Extraction Ratios (%)
	Arterial	Δ	Arterial	Δ	Arterial	Δ	Arterial	Δ	Arterial	Δ	Arterial	Δ	
(A) 17	100.1	1.5	0.356	0.036	12.51	3.56	5.32	0.19	0.747	0.710	0.154	77.06
(B) 17	334.0	12.0	0.280	0	6.80	0.14	5.88	0.21	1.140	0	1.250	0.50	75.48
(C) 17	102.1	7.1	0.336	0.037	11.69	4.61	5.30	0.07	1.190	0.02	1.190	0.315	52.38
(A) 18	81.0	2.1	0.389	0.063	6.17	3.13	4.40	0.34	0.797	0.007	0.458	0.312	63.17
(B) 18	278.0	8.8	0.389	0.018	4.95	0.15	4.42	0.32	1.405	0.015	2.96	1.65	68.63
(C) 18	87.6	4.4	0.354	0.07	9.17	0.57	2.80	0.16	1.29	0.06	0.917	0.524	78.0
(A) 19	103.6	3.4	0.496	0.202	12.52	5.70	4.77	0.41	1.4	0	0.368	0.092	103.75
(B) 19	268.4	6.2	0.432	0.072	7.03	0.67	3.88	0.10	1.52	0	3.58	1.58	42.16
(C) 19	94.8	4.2	0.360	0.0	8.24	0.90	2.99	0.41	1.37	0.005	0.715	0.071	11.9
(A) 28	93.8	3.8	0.470	0.168	9.03	4.31	4.32	0	1.095	0.016	0.885	0.295	54.44
(B) 28	232	6.4	0.334	-0.016	6.10	1.04	5.38	0.22	1.190	0.015	1.28	0	78.85
(C) 28	81.3	7.7	0.683	0.3	16.2	5.8	4.05	0	1.2	0.033	1.12	0.213	70.02
(A) 29	103.6	3.4	0.608	0.312	8.09	3.98	4.88	0.17	0.6	0.022	1.536	0.108	108.35
(B) 29	338	13.4	0.710	0	9.55	0.8	5.5	0.1	1.15	0	9.14	2.12	45.48
(C) 29	164.9	8.6	0.423	-0.337	11.40	-2.15	3.7	0.1	1.02	0.015	1.468	0.448	97.71
(A) 32	95.6	5.4	0.355	0.017	9.06	3.62	5.0	0.18	0.674	0.017	0.84	0.084	6.43
(B) 32	361.2	9.2	1.505	0.363	18.24	1.74	9.8	0.2	1.38	0.04	13.12	4.0	86.32
(C) 32	349.0	15.0	1.273	-0.927	35.0	2.0	7.1	0.3	1.29	0.015	3.88	1.02	127.62
(A) 33	88.2	3.5	0.736	0.324	13.7	7.35	4.65	0.20	0.525	0.018	0.935	0.135	236.55
(B) 33	246.2	4.2	1.039	0.257	7.68	-0.44	7.7	0	1.210	0.02	14.32	3.26	115.3
(C) 33	242.0	4.8	0.646	0.152	9.02	0	5.6	0.07	0.95	0.017	6.5	2.3	106.54
(A) 40	86.7	5.1	0.607	0.213	10.52	4.70	4.70	0.12	1.130	0	0.990	0.130	8.98
(B) 40	245	3.4	0.591	0.085	8.00	1.64	3.96	0.02	1.590	0.044	7.32	2.28	267.52
(C) 40	113.8	2.8	0.490	-0.186	10.8	0.92	2.57	0.06	1.375	0.015	2.14	0.28	100.98
(A) 41	93.0	7.8	0.448	0.032	6.19	3.04	4.55	0.05	0.847	0.010	0.427	0	59.31
(B) 41	484	4.0	0.783	0.083	8.93	0.13	9.35	0.15	1.536	0.020	5.74	1.5	121.29
Mean													
(A and B)	+215	+3.51	+0.169	-0.56	-1.17	-3.72	+1.475	+0.037	+0.488	+0.007	+5.815	+1.7195	-18.143
Range (A and B)	158-391	-1.7 to 10.5	0 to +0.335	-0.036 to +0.346	-6.06 to +0.918	-7.79 to -1.88	-0.89 to +4.80	-0.31 to +0.22	+0.095 to +1.206	+0 to +0.044	+3.46 to +13.395	+1.7195 to +3.916	+59.131 to +51.96
(B and C)	-140.54	-1.77	-0.094	-0.194	+5.27	+0.39	-1.76	0.015	-0.167	-0.0037	-4.376	-1.302	-37.204
Range (B and C)	-231.9 to -4.2	-4.9 to +5.8	-0.393 to +0.349	-1.29 to +0.316	+1.21 to +16.76	-2.95 to +4.76	-3.65 to -0.58	-0.22 to +0.31	-0.59 to +0.05	-0.029 to +0.045	-2.98 to -0.060	-1.302 to +0.213	-51.34 to +56.11

(A) = control dogs; (B) = control dogs made diabetic by alloxan; (C) = diabetic dogs given insulin intravenously.

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TABLE III

Blood Levels and Myocardial Extractions											
	Coronary Flow	Arterial O ₂ (vol. %)	Glucose (mg. %)	Pyruvate (mg. %)	Lactate (mg. %)	Amino Acids (mg. N/100 cc.)	Fatty Acids (mEq./100 cc.)	Ketones (mg./100 cc.)	Arterial Δ	Arterial Δ	Arterial Δ
<i>Statistical Analyses of the Differences Obtained by Comparison of Human Diabetics and Non-Diabetics</i>											
t	1.6	4.85	1.8	1.6	2.29	0.966	2.38	1.46
P	>0.1	>0.1	<.05	>0.1	<.005	<0.1 >.05	>0.1	<.005 >.01	>0.1	<.05 >.01	>0.1
Conclusions	N.S.	N.S.	S	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
<i>Statistical Analyses of the Differences Obtained by Comparison of the Control and Alloxan-diabetic Dogs</i>											
t	2.34	2.02	2.22	1.16	.92	1.41	6.41	2.02	.07	3.614
P	<.05 >.01	>.1	<.005	<.1 >.05	>.1	>.1	>.1	<.005	<0.1 >.05	>.1	<.05 >.01
Conclusions	S	N.S.	S	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S
<i>Statistical Analyses of the Differences between the Alloxan-diabetic Dogs, and the Alloxan-diabetic Dogs Given Insulin</i>											
t	1.75	5.25	1.5	1.29	1.88	.11	5.64	2.737
P	>.1	>.1	<.005	>.1	>.1	>.1	<.05 >.01	>.1	<.005	>.1	>.1
Conclusions	N.S.	N.S.	S	N.S.	N.S.	N.S.	S	N.S.	S	N.S.	S
<i>Myocardial Usage (100 gm. left ventricle per min.)</i>											
	O ₂ vol. (%)	Glucose (mg.)	Pyruvate (mg.)	Lactate (mg.)	Amino Acids (mg. N)	Fatty Acid (mEq.)	Ketone (mg.)	Glucose	Pyruvate	Lactate	Amino Acids
<i>Statistical Analyses of the Differences Obtained by Comparison of Human Diabetic and Non-Diabetics</i>											
t	1.96	1.91	2.5	2.78	0.974	1.95	1.77	2.34
P	>0.1	<.10 >.05	<.05 >.01	>0.1	<.05 >.01	>0.1	<.01	<0.1 >.05	<.05 >.01	>0.1	<.05 >.01
Conclusions	N.S.	N.S.	N.S.	S	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S
<i>Statistical Analyses of the Differences Obtained by Comparison of the Control and Alloxan-diabetic Dogs</i>											
t	.705	1.75	.37	2.67	1.15	1.54	3.457	1.501	1.07	3.105
P	>.1	<.1	>.1	<.05	>.1	>.1	<.01	>.1	<.005	>.1	<.05 >.01
Conclusions	N.S.	N.S.	N.S.	S	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
<i>Statistical Analyses of the Differences between the Alloxan-diabetic Dogs, and the Alloxan-diabetic Dogs Given Insulin</i>											
t	.85	1.55	1.43	3.855	.257	1.65	2.38
P	>.1	>.1	>.1	>.1	>.1	>.1	>.01	>.1	>.1	>.1	>.1
Conclusions	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

S = Significance (acceptable as statistically significant only at the 5% level ($P = .05$)); N.S. = Not significant.

series may indicate that the diabetes was mild or well controlled. Myocardial extraction and usage of amino acids was similar to that found in normal individuals.¹⁹ There was an elevation in myocardial usage of ketones in diabetic patients (0.96 as compared to 0.58 mg./min./100 gm. left ventricle, Table I). The probability value, however, was 0.1, reducing the significance of this observation. (Table III.)

Results Obtained on Dogs. The changes in cardiac metabolism occurring subsequent to the development of alloxan diabetes in dogs are illustrated in Tables II and III. As compared to the normal animal there was a significant rise in arterial levels of glucose, fatty acids, ketones and amino acids. (Table II.) The severity of the diabetes is illustrated by a mean rise in arterial glucose and ketone concentrations of 215 and 5.8 mg. per cent, respectively. (Table II.) The mean rise in arterial concentration of fatty acids was also considerable (0.48 mEq., Table II). This was probably the result of increased mobilization from fat depots.¹⁶ High amino acid concentrations in diabetes were also found by Luetscher,^{1,20} probably the result of deficient protein synthesis.

Myocardial usages of ketones and of glucose were increased. The usage of lactate was diminished (Table III) and the myocardial usage of pyruvate was slightly depressed. Apparently, as in the case of the diabetic human heart, glucose was utilized by the diabetic dog heart, and the myocardial usage and extraction of lactate was diminished. However, in contrast to results obtained with the human heart, the diabetic dog's heart failed to use an increased quantity of fatty acids despite elevated fatty acid blood levels. As a result of the diminution in lactate utilization, there was a mean fall in oxygen extraction ratio of all carbohydrate material of 18.1 per cent. (Table II.) The oxygen extraction ratio of non-carbohydrate material, on the other hand, showed a mean rise of 59 per cent due to increased myocardial uptake of ketones and fatty acids. (Table II.) Therefore, as in the human heart, the reduction in the total myocardial carbohydrate utilization was compensated for by an increased usage of non-carbohydrate material.

Insulin caused the expected significant fall in blood sugar (mean fall of 140 mg. per cent, Tables II and III). Despite this, the myocardial usage and extraction of sugar did not rise. This was surprising since it had been generally

assumed that insulin provokes an increase in the rate of glucose utilization.³ The majority of workers found an increase in utilization of glucose by the isolated heart subsequent to insulin, although some investigators found no change.³⁹ Increased glucose consumption following insulin has also been demonstrated in the isolated rat diaphragm.⁴⁰

In addition to a fall in arterial levels of glucose, insulin caused a decline in arterial concentration of fatty acids, amino acids and ketones. (Table II.) As in the case of glucose, the myocardial usage of these compounds showed no significant change. (Table III.) Only in the case of ketone bodies was the decline in arterial concentration accompanied by a change in their myocardial usage and extraction, which showed a decline after insulin of 1.3 mg. per cent. (Table II.) This was statistically significant (p of 0.01, Table III). Table II illustrates that the blood concentrations of lactate increased following insulin (mean rise of 5.3 mg. per cent).

Myocardial usage of lactate remained unchanged. This finding is in contrast to that obtained by Evans⁷ who found that in the heart-lung preparation the increased myocardial glucose utilization resulting from insulin was partly compensated by a fall in myocardial lactate consumption. Increased arterial concentrations of lactate and pyruvate were observed following insulin injections by Chesler and Himwich⁴¹ in depancreatized dogs. Table III illustrates that blood pyruvate concentrations showed no significant change following insulin.

Although there was no statistically significant change in myocardial usage or extraction of pyruvate (probability value of .1, Table III), a negative myocardial pyruvate balance occurred in four animals following the injection of insulin. (Table II.) It is likely, according to Bueding,¹³ that the increased concentration of pyruvate in blood following insulin results from its increased production rather than decreased removal. On the other hand, if insulin increases the carboxylase content of the heart, as occurs in the liver and blood of diabetic animals, myocardial removal of pyruvate should be increased.⁴² It is likely that the heart differs in this respect from other organs studied.

COMMENTS

The results reported in this paper demonstrate a series of metabolic alterations occurring in the heart of patients with diabetes mellitus and of

dogs with alloxan diabetes. In both patients and dogs, the mean value for myocardial usage of carbohydrates is reduced and the utilization of non-carbohydrate material is increased. This is particularly conspicuous in the human subjects. (Table I.) Apparently the heart is not exempt from the most important metabolic defect in diabetes, that of deficient utilization of carbohydrates.^{3,4}

Deficient myocardial glucose utilization has also been noted in the isolated heart *in vitro*. Evans⁷ found that the diabetic dog's heart consumed only one-fourth of the glucose utilized by the non-diabetic organ. Cruickshank⁸ found that the diabetic heart perfused with blood from diabetic animals does not utilize sugar. It had been shown in previous reports that in the normal human heart the myocardial extraction and usage of glucose increases with rising arterial blood concentrations.²⁴ At blood concentrations exceeding 110 mg. per cent, myocardial glucose extraction reached its maximal values.²⁴ The range of myocardial glucose extraction in non-diabetic man is from 0.9 to 15.0 mg. per cent glucose at blood levels ranging from 74 to 135 mg. per cent. (Table I.) In the diabetic this range is from 0.06 to 5.7 mg. per cent with much higher arterial glucose concentrations (from 78 to 254 mg. per cent, Table I.). In one patient (A. M. M.) in whom the arterial glucose concentration was 265 mg. per cent, the myocardial extraction was only 5.7 mg. per cent. (Table I.) In the non-diabetic heart it probably would have exceeded 10 mg. per cent.²⁴ Thus, although the human diabetic heart can utilize glucose, it does so in diminished amounts.

The blood glucose concentrations in diabetic dogs ranged from 232 to 484 mg. per cent, illustrating the greater severity of the disease as compared with the patients. (Table II.) The myocardial glucose extraction in these animals ranges from 3.4 to 13 mg. per cent as compared to 1.5 to 7.8 in the normal dogs. (Table II.) This indicates that the heart of the diabetic dog at its hyperglycemic level utilizes as much sugar as the normal dog at its usual normal blood sugar level. However, a relative deficiency in myocardial glucose usage is probably present, since the non-diabetic heart of dogs extracts significantly greater quantities of sugar at equivalent blood sugar concentrations.⁵⁰

The non-diabetic human heart extracts considerable quantities of lactate from the blood, the relationship between lactate extraction and

arterial lactate concentration depending upon the latter.²⁴ The myocardial lactate usage of both the diabetic human and dog's heart is significantly reduced. (Tables I to III.) This is in contrast to results obtained by Evans et al.⁷ who found almost unimpaired utilization of this foodstuff by the isolated heart. They concluded that usage of lactate replaces or supplements that of sugar in the diabetic heart. There is no evidence for this in the results presented in this report. On the contrary, the marked reduction in myocardial usage of lactate is primarily responsible for the reduction in the total amount of energy available from the carbohydrate fraction. (Tables I and II.) Some evidence exists that the diabetic organism as a whole is deficient in its use of lactate. Chambers et al.⁴³ found that the arterial lactate concentration of depancreatinized dogs reached higher values on exercise than was observed in non-diabetic animals, suggesting diminished utilization of lactate.

Pyruvate is metabolized by the non-diabetic human heart, the myocardial extraction ranging from 0 to 0.77 mg. per cent with a mean of 0.21. (Table I.) The myocardial pyruvate extraction of diabetic human hearts ranges from 0.03 to 0.22 with a mean of 0.1 (Table I.) Although this fall itself is statistically not significant, it assumes importance when one considers that it occurs in the presence of a significant elevation in arterial concentration of pyruvate. (Tables I and III.) This suggests some deficiency in myocardial utilization of pyruvate. The diabetic dog's heart also exhibits some interference with pyruvate utilization. There is a fall in mean values for myocardial extraction and usage of pyruvate as compared with the normal of 0.56 mg. per cent and 0.027, respectively. (Table II.) Deficient utilization of pyruvate has also been described by Shorr,¹⁰ who concluded that tissue slices from diabetic dogs utilize pyruvate and lactate less readily than those from normal animals. In line with this are the results of Pearson et al.¹¹ who found diminished pyruvate utilization in both cardiac and diaphragmatic muscle of diabetic animals. Villee and Hastings⁴⁴ discovered that the rates of oxidation of acetate and pyruvate to CO₂ were diminished in diaphragms from alloxan-diabetic rats. It is possible that there is a metabolic block between pyruvic acid and tricarboxylic acids, since a diminution of the tricarboxylic acids of the Krebs cycle was found in the presence of essentially normal lactic and pyruvic acid concentrations.⁴⁵ Defective phos-

phorylation of thiamine to cocarboxylase in the diabetic animal may be responsible for this block.^{14,15}

The human heart in patients with diabetes mellitus extracts a significantly greater amount of fatty acids than does the non-diabetic heart. (Tables I and III.) In the normal subject the myocardial fatty acid extraction ranges from 0.002 to 0.03 mEq. with a mean of 0.0127 mEq. (Table I.) In the diabetic the range of fatty acid extraction is from 0.02 to 0.19 mEq. with a mean of 0.071 mEq. (Table I.) (Probability value of this difference 0.01, Table III.) It has been shown that the normal heart can store fat, since ingestion of fat resulted in oxygen extraction ratios of over 100 per cent.¹⁹ Since the oxygen extraction ratios of fatty acids in diabetic hearts has a mean value of 293 per cent, with a range of from 86 to 600 per cent, the likelihood of storage of fatty acids by the diabetic heart must also be considered. (Table I.) In contrast to the normal heart in which storage usually occurs only when the fatty acid concentration in blood is increased, the diabetic heart appears to store fatty acids at their normal blood concentration. In the diabetic dog, on the other hand, despite significant increase in concentration of arterial fatty acids, myocardial extraction of fatty acids is not altered. (Mean rise of 0.007 mEq. with a probability value of 0.1, Tables II and III.) The reason for this discrepancy is not clear. Severity of the disease, species differences and differences in type of diabetes may be responsible. Increased myocardial utilization of fatty acids, as observed in human diabetes, is consistent with results obtained on the isolated heart *in vitro*.³⁹ One may venture the opinion that there is some connection between this large myocardial extraction of fatty acids and the disability of the diabetic organism in synthesizing them from glucose, lactate or pyruvate.¹⁷ Since fatty acids cannot be synthesized and the usage of carbohydrate is limited, their storage represents a stabilizing reserve to be drawn upon by the heart when the necessity arises.

The usage of ketone bodies of hearts of diabetic patients is slightly increased. (Table I.) The hearts of dogs made diabetic with alloxan consume a much greater quantity of ketones than does the normal animal. (Mean difference between the control and the diabetic animal of 1.81 mg./100 gm./min. and a probability value of 0.01, Table III). The increased myocardial usage of ketones of the dog's heart occurs in

the presence of elevated arterial ketone concentrations. (Table II.) In the heart of non-diabetic individuals aerobic metabolism of ketones accounts for approximately 5 per cent of the total oxygen extraction of the heart.¹⁹ In diabetic patients this figure is 9.8 per cent. (Table I.) The contribution of ketones to the aerobic metabolism of the diabetic dog's heart is 13.5 per cent higher than that of the normal heart. It has been shown in a previous publication that there is a negative correlation between myocardial carbohydrate and ketone utilization, indicating that carbohydrates inhibit ketone utilization by the heart, a finding which is in accord with the theory of substrate competition for available oxygen.¹⁹ Waters et al.⁴⁶ demonstrated in the heart-lung preparation that when both carbohydrate and ketone bodies are available, the former is used in preference to the latter. Therefore, the decrease in the rate of total oxidation of carbohydrates by the heart may have been responsible for the increased myocardial usage of ketones.

The arterial concentration of amino acids and their myocardial extraction and usage are normal in diabetic patients. (Tables I and III.) In diabetic dogs the amino acid concentrations in arterial blood are slightly elevated but their myocardial extraction and usage are not significantly altered. (Tables II and III.) It has been shown that amino acids are extracted in considerable quantity from the coronary blood of normal individuals.¹⁹ After infusion of amino acids as much as 40 per cent of the total cardiac oxygen consumption can be accounted for by their aerobic metabolism.¹⁹ A small rise in arterial amino acid concentrations of 20 per cent produced a disproportionate increase of 245 per cent in their myocardial extraction. The rise in amino acid concentration in the blood of diabetic dogs is 32 per cent, with an increase in myocardial extraction of amino acid of only 4 per cent. The possibility exists that the failure of the diabetic heart to respond to an elevation in arterial amino acid concentration with a rise in their myocardial extraction represents a deficiency in protein synthesis.²¹ Krahl studied the incorporation of glycine-1-C14 into liver glutathione and protein fractions of the liver in the normal rat and in the alloxan-diabetic rat. He found that there is a direct dependence of glutathione synthesis in liver slices upon availability of carbohydrate intermediates.

Insulin injection into diabetic dogs results in a

significant fall in blood sugar (mean decline of 140.5 mg. per cent, Table II). The myocardial usage and extraction of glucose, on the other hand, fails to increase. (Table III.) Although there is disagreement on the theories advanced to explain insulin action, it is generally agreed that the hormone favors glucose utilization.¹ For example, Feller⁴ found that in dogs totally devoid of insulin-secreting tissue, the rate of glucose oxidation was far below normal. Wick et al.,⁴⁷ using C-14 labeled glucose, found that administration of insulin resulted in a fourfold increase of CO₂ derived from the labeled glucose. In the heart-lung preparation it was found that addition of insulin to the perfusate results in restoration of utilization of glucose by the heart.³⁹ On the surface, therefore, failure of insulin to produce increased myocardial glucose usage might suggest that the hormone has no effect on glucose utilization by the diabetic heart. When one considers, however, that in the normal heart myocardial extraction and usage of sugar decrease with a decline in arterial glucose concentration, the findings here reported would imply that insulin increases myocardial glucose utilization.^{24, 50}

It has already been mentioned that both diabetic patients and diabetic animals exhibit some interference with myocardial pyruvate usage and extraction. The thought was expressed that this might be the result of a metabolic block between pyruvic acid and the tricarboxylic acid cycle.⁴⁵ Accordingly, insulin should increase the oxidation of pyruvate. This is apparently not the case. On the contrary, the pyruvate concentration in coronary vein blood exceeded that in coronary arterial blood in four animals following the injection of insulin. (Table II.) Similar "pyruvate reversals" were previously encountered during hemorrhagic shock.⁴⁸ The reason for this is not clear. Heart muscle may differ from other organs with respect to the effect of insulin on pyruvate oxidation. Purvate reversal may also be an indication of increased catabolism of endogenous carbohydrate in heart muscle, initiated by a rapidly falling blood sugar level in these severely diabetic animals. Under these conditions the block between pyruvate and the Krebs cycle may persist because of a reduced thiamine content in heart muscle, insufficient for the formation of co-carboxylase. Pearson et al.¹¹ found that, in contrast to the diabetic diaphragm, the addition of insulin *in vitro* had no significant effect on

the utilization of pyruvate in either normal or diabetic heart slices.

If insulin corrects the metabolic defect responsible for diminished lactate usage and extraction in the heart of diabetic patients and dogs, its injection should result in increased myocardial utilization of lactic acid. The evidence for this is lacking. The data in Table II indicate that there is a significant increase in arterial lactate concentration; its myocardial usage and extraction, however, rises but little. Chesler and Himwich⁴¹ observed an increase in arterial lactic acid concentration following insulin. In their experiments, however, arterial pyruvate levels were also elevated, suggesting to these workers that only pyruvate is formed as a direct result of insulin, while the concomitant rise in lactic acid occurs only indirectly because of an equilibrium between the two metabolites.

Insulin causes a fall in the blood concentration of fatty acids without significantly affecting their myocardial usage or extraction. (Tables II and III.) It is likely that the fall in blood concentration is due to decreased mobilization from fat depots. Diabetic animals exhibit marked impairment of fat synthesis.¹⁷ Brady and Gurin¹⁸ have shown that insulin does not reverse this metabolic defect in the diabetic liver. This finding, if it applies also to the heart, might explain the tendency of heart muscle to store fatty acids for use in energy production, despite the presence of insulin.

Insulin causes a diminution in arterial concentration of ketones and their myocardial usage and extraction. (Tables II and III.) The mean decline in the contribution of ketones to the aerobic metabolism of the heart following insulin is approximately 10 per cent. It is possible that myocardial ketone utilization is governed by the arterial ketone concentration. The fall in arterial concentration of amino acids resulting from insulin is not accompanied by significant changes in their myocardial usage or extraction. (Tables II and III.) A return of blood levels of amino acids to normal following insulin has also been observed by Luetscher.²⁰ This is probably the result of increased synthesis of proteins.²¹ Whether protein synthesis in heart muscle remains unaffected by insulin cannot be ascertained from these experiments.

The results reported in this paper indicate that many of the metabolic alterations described are present both in the diabetic dog and in man. Common features are a reduction in myocardial

lactate usage and a slight decline in that of pyruvate. There is no change in utilization of amino acids by the heart in both species. Myocardial glucose consumption is reduced in dog and man relative to the elevation in blood glucose concentration. The myocardial usage of ketones is slightly increased in diabetic hearts of patients and significantly elevated in the dog. The main difference concerns the utilization of fatty acids; this is significantly increased in the human heart but is unchanged in the dog. Whether this is due to a species difference or to differences in type and severity of diabetes is not clear. Anesthesia, which was used in the dogs, may have played some part.

When compared with results obtained in studies on the myocardial metabolism of non-diabetic man and dog, a broad spectrum of metabolic changes is found in the diabetic heart probably resulting from varying degrees of interference with glucose utilization.¹ Thus the diabetic heart is not deficient only in glucose utilization—the metabolism of the other carbohydrates (pyruvate and lactate) is also affected. In addition, the defect extends to the metabolism of proteins and fat. In the light of the present concept of hypoinsulinism, it is not possible to state whether disturbed insulin-enzyme interaction responsible for these alterations can be explained upon the basis of a single mechanism or whether they are the result of the effect of this hormone on a variety of cellular reactions.

SUMMARY

Changes occurring in the metabolism of the heart in patients with diabetes mellitus and in dogs with alloxan diabetes have been investigated, using the technic of coronary sinus catheterization.

The myocardial glucose usage of the diabetic heart was diminished relative to the normal heart.

There was a marked decrease in usage of lactate by both the human and the dog heart. Myocardial usage of pyruvate was slightly diminished.

Myocardial usage of ketones was slightly increased in diabetic patients and significantly elevated in diabetic dogs.

The myocardial fatty acid extraction in diabetic patients was significantly increased; it was normal in the diabetic animals.

Insulin injection into diabetic dogs was with-

out effect on myocardial glucose extraction. This suggests a relative increase in myocardial glucose utilization, since the arterial glucose concentration declined. Insulin in several instances caused a negative pyruvate extraction by the heart. The hormone had no effect on the myocardial usage of lactate, despite significant elevations in arterial lactate concentration.

Arterial concentrations of fatty acids diminished following insulin without changes in myocardial usage and extraction of fatty acids. Insulin caused a diminution in arterial concentration of ketones and their myocardial usage.

The consumption of amino acids by the diabetic heart remained unaffected by insulin despite a fall in arterial amino acid concentration.

The results indicate that the diabetic heart is deficient in the metabolism of carbohydrates, proteins and fats.

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Ventricular Function*

VII. Changes in Coronary Resistance and Ventricular Function Resulting from Acutely Induced Anemia and the Effect Thereon of Coronary Stenosis

ROBERT B. CASE, M.D., †‡ ERIK BERGLUND, M.D., and STANLEY J. SARNOFF, M.D.†
Boston, Massachusetts

THE response of the circulatory system to anemia is manifested by significant changes among which are tachycardia, increased cardiac output, a fall in peripheral resistance and cardiomegaly.¹ This communication will deal with the extent to which coronary vessels participate in the response to anemia and compensate for the reduced oxygen carrying capacity of the blood. The effects of anemia on the myocardium will also be analyzed by the use of ventricular function or modified Starling curves.² Data will be introduced which indicate that the functional capacity of the myocardium is limited by the extent to which the coronary vessels can provide compensatory dilatation. The limitation placed by coronary stenosis on the ability of the coronary vessels to compensate for anemia will also be presented and the possible clinical interrelationship of these two diseases will be discussed. Preliminary reports have been made elsewhere.^{3,4}

METHOD AND MATERIAL

Dogs under morphine-chloralose-urethane anesthesia, and with constant volume positive pressure breathing, had ribs 3, 4 and 5 resected on the left. Bilateral high cervical vagotomy was performed in seven of the eight dogs studied. The manner in which the circulation was then rearranged is described elsewhere.⁵ Briefly, the blood leaving the left ventricle enters the aorta from which it flows through a Potter electroturbinometer⁶ to the descending aorta and the brachiocephalic artery. A sidearm proximal to the

electroturbinometer leads a portion of the blood through a recording rotameter;⁷ from this, blood flows to a modified Gregg coronary cannula the tip of which is secured in the lumen of the left main coronary artery by a previously placed silk ligature. Thus the total cardiac output was metered except for blood entering the right coronary artery (approximately 1 per cent of cardiac output).⁸ Pressures were measured in the left and right atria, and in the pulmonary artery and aortic arch by means of electro-manometers. Pressures and flows were continuously recorded on a direct-writing oscillograph. Clotting was prevented with treburon.⁹ The filling pressures of both ventricles were increased in a stepwise fashion by intermittently allowing blood to flow into the right femoral vein from a reservoir. In this way data were gathered for the calculation of right and left ventricular function curves, and peripheral, pulmonary and coronary vascular resistance values. Data were then obtained for successive grades of anemia. These were produced by replacing a portion of the blood in the reservoir with 6 per cent dextran¹⁰ in saline and mixing thoroughly with the blood in the animal.

Ventricular stroke work in gram meters was calculated by multiplying stroke volume in cubic centimeters by the difference between mean arterial and mean atrial pressure in

§ Supplied by Dr. Elmer Sevringshaus, Hoffman-La-Roche, Inc., Nutley, N. J.

|| Expandex supplied by Mr. Jerome Martin, Commercial Solvents Corporation.

* From the Department of Physiology, Harvard School of Public Health, Boston, Mass. Supported by grants-in-aid from the United States Public Health Service, Bethesda, Maryland, the Life Insurance Medical Research Fund, New York, N. Y. and Sharp & Dohme, Inc., Philadelphia, Pa. Presented before the Department of Medicine, University of London, Postgraduate School of Medicine, February 1954.

† Research Fellow, National Heart Institute, United States Public Health Service.

‡ Present address: National Heart Institute, National Institutes of Health, Bethesda, Md.

centimeters of water and dividing by 100. Right and left ventricular function curves were obtained by plotting the stroke work of each ventricle against its simultaneous mean atrial pressure over the whole range of atrial pressures obtained. (Figs. 4 and 5.) The reader is referred

Eight dogs were studied. In seven of these severe anemia was produced; in the remaining dog the effect of moderate anemia and coronary stenosis was studied.

In two experiments the oxygen consumption of the myocardium supplied by the left coronary

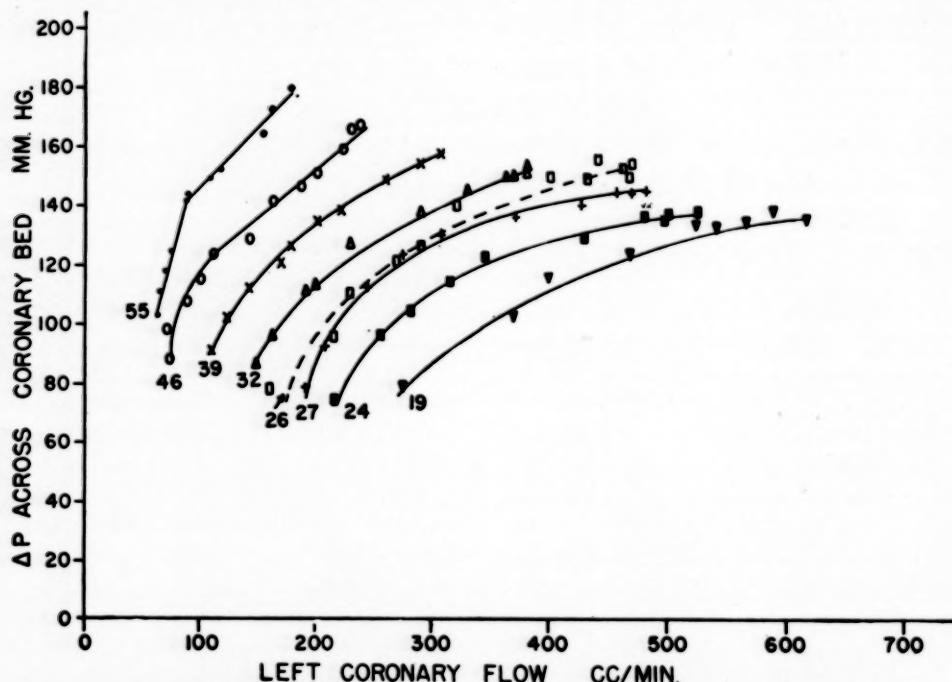


FIG. 1. Pressure flow relationships in the coronary bed at various hematocrits. Experiment No. 104; dog weight 29.0 kg.; pericardium intact; vagotomy. Pressure gradient in mm. Hg across coronary bed plotted against left coronary flow in cc. per minute. Hematocrits represented by numbers at the bottom of each curve. Dotted line is curve obtained after reinfusion of red cells.

to a previous publication for further details and an evaluation of the method.²

Resistance values were calculated⁹ by dividing the pressure drop across the vascular bed in question by the flow through it and expressed as

$$\frac{\text{mm. Hg}}{\text{cc. per min.}}$$

For the coronary resistance, the pressure drop was taken to be the pressure at the coronary ostium minus the mean right atrial pressure. Pressure at the coronary ostium was calculated by subtracting from the aortic pressure the previously determined pressure drop across the rotameter-tubing-cannula system for the concomitant coronary flow. Similarly, the pressure drop across the peripheral bed was determined by subtracting right atrial pressure from the pressure existing distal to the electroturbinometer.

artery was calculated from the recorded flow therein and the oxygen difference between simultaneous arterial and coronary sinus samples. The latter¹⁰ were obtained through a fluoroscopically placed catheter.* The technic of Van Slyke and Neill¹¹ was used for blood oxygen analysis.

RESULTS

Effect of Anemia on the Pressure-Flow Relations in the Coronary Vascular Bed. At any given hematocrit the ventricular filling pressure, cardiac output, coronary flow, aortic pressure and ventricular work were elevated in a stepwise fashion by the intermittent blood infusions as described. From these the relationship between the pressure gradient across the coronary bed and the left coronary flow could be plotted.

* Grateful acknowledgment is made to Dr. Walter T. Goodale for the demonstration of this technic.

Figure 1 shows the effect of varying degrees of acutely induced anemia on this relationship. It may be seen that the coronary flow at any given pressure gradient is substantially increased when the hematocrit is lowered. For example, at a hematocrit of 55 per cent a pressure gradient

Change in Coronary Resistance Resulting from Anemia. The calculated coronary resistance is plotted against the perfusion pressure at the coronary ostium in Figure 2. Three things are noteworthy in this plot. First, the coronary resistance undergoes a great change with

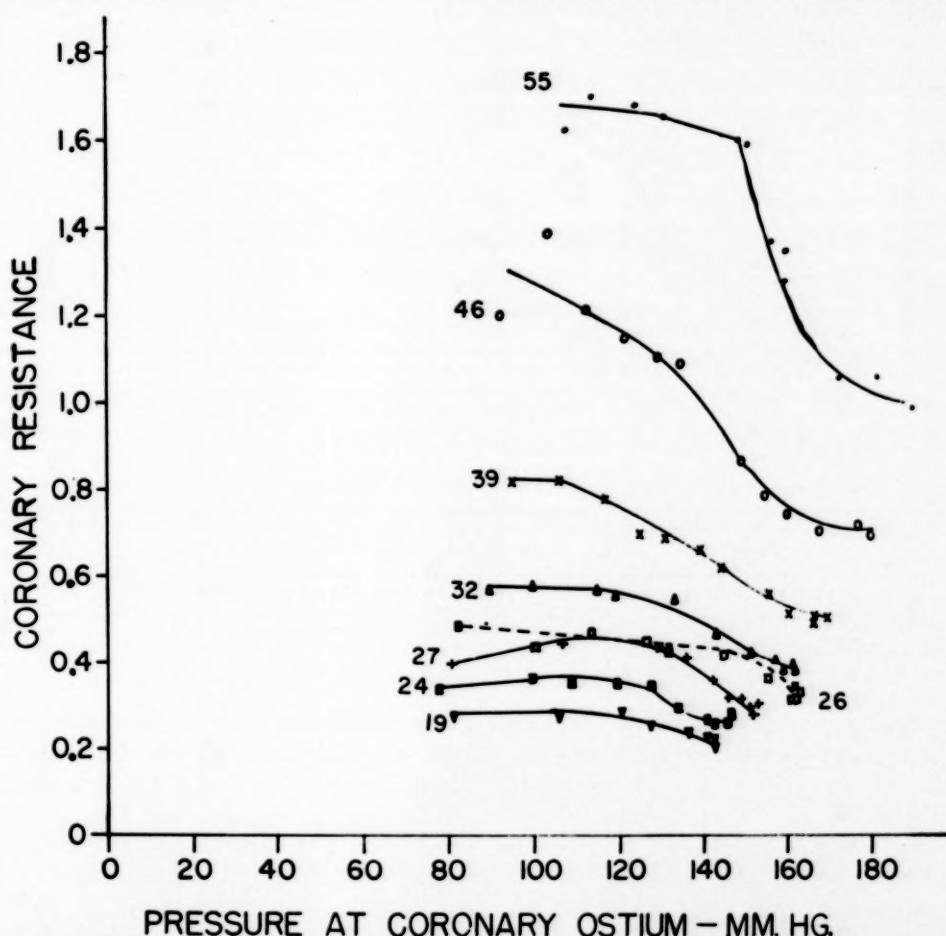


FIG. 2. Changes in coronary resistance with anemia. Experiment No. 104; coronary resistance expressed as mm. Hg/cc./min. Numbers at left of curves indicate hematocrit, and dotted line represents values obtained after reinfusion of red cells.

of 110 mm. Hg across the coronary vascular bed produced a blood flow of 65 cc. per minute; at a hematocrit of 19 per cent this same pressure gradient resulted in a flow of 402 cc. per minute, an increase of over 500 per cent. At higher pressure levels even greater increments in flow may be seen. At the end of the experiment the hematocrit was raised from 19 to 26 per cent by the addition of centrifuged red cells previously removed from the reservoir. The resulting values are indicated by the dotted line and are close to the values obtained previously at 27 per cent. Changes similar to those in Figure 1 were obtained in all eight experiments.

anemia from a high of 1.7 at a hematocrit of 55 per cent to 0.28 at a hematocrit of 19 per cent. The reduction of resistance at any given perfusion pressure which occurs during anemia indicates the extent to which the tone of the coronary vessels has diminished. This reduction in tone with anemia is marked at first and then becomes progressively smaller, suggesting that the limit of dilatation of the coronary bed is being reached. Lastly, at the higher hematocrits there is a decrease in coronary resistance as the work and coronary perfusion pressure is increased. This is a further indication of the magnitude of the vasodilatory reserve at higher hematocrit

levels. At hematocrits below 32 per cent there is little change in resistance as coronary perfusion pressure is increased indicating a lower vaso-dilatory reserve. Changes similar to those depicted in Figure 2 were obtained in all eight animals.

At the top of Figure 4 are left ventricular function curves obtained by plotting left ventricle stroke work against mean left atrial pressure; also shown are mean aortic pressure, left main coronary artery flow, and stroke volume, plotted against mean left atrial pressure.

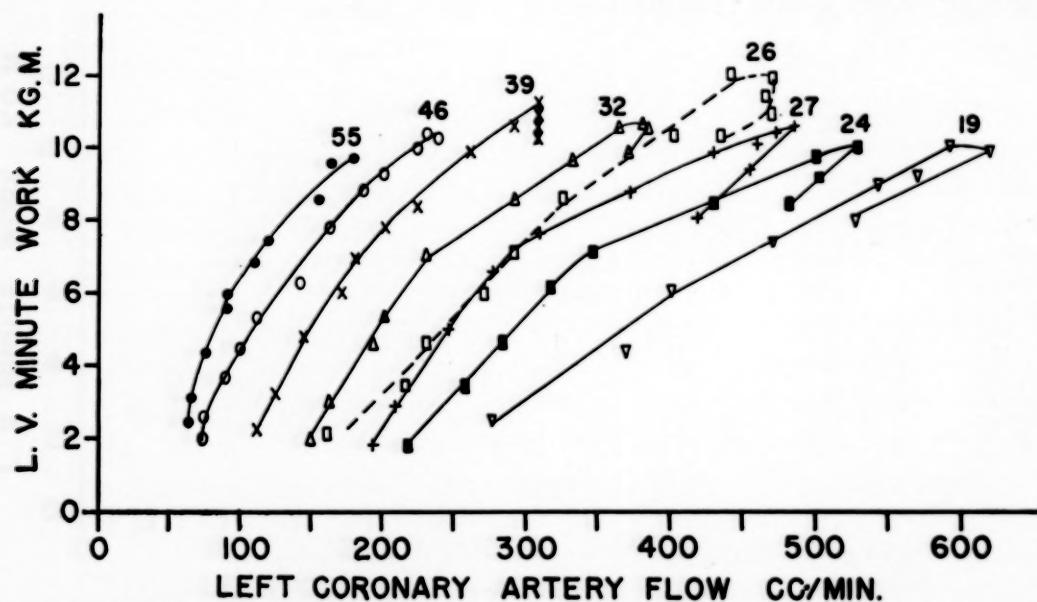


FIG. 3. Work-flow relationships during anemia. Experiment No. 104. Left ventricular work in kg.-M. plotted against left coronary flow in cc. per minute. Figures above each curve represent hematocrits; the dotted line is the curve obtained after the reinfusion of red cells.

Effect of Anemia on the Relation Between Ventricular Work and Coronary Flow. At any given hematocrit there is a clear relationship between ventricular work and coronary flow. (Fig. 3.) As the hematocrit is decreased there is an increased coronary flow at any level of left ventricular work. For example, when the left ventricle was performing an external work of 5 kilogram-meters per minute, the left coronary flow was 83 cc. per minute at a hematocrit of 55 per cent. At the same work level coronary flow was 193 cc. at a hematocrit of 32 per cent and 370 cc. at a hematocrit of 19 per cent. Of further interest is the change in the slope of these curves as the hematocrit is decreased. Changes similar to those depicted in Figure 3 were obtained in all eight experiments.

The lines in Figure 3 denote the order in which the points were obtained. At high work levels, in the more anemic state, there was a fall in work per unit of coronary flow. This was usually associated with a descending limb on the ventricular function curve. The significance of this finding is not clear.

Effect of Anemia on Ventricular Function Curves.

The ventricular function curves at hematocrits of 43 per cent and 32 per cent are practically identical and do not have a descending limb. When the hematocrit has reached 24 per cent, however, a depression of the curve does occur, that is, less work is obtained per unit of filling pressure. At a hematocrit of 17.5 per cent marked depression occurs, with a frank descending limb at higher filling pressures. In another similar experiment reinfusion of centrifuged red cells returned the depressed ventricular function curve to a higher level and abolished its descending limb. In five of the seven experiments in which the effect of severe anemia was studied the results were similar to those shown in Figure 4, with depression of the ventricular function curve first being noted at hematocrits ranging from 24 to 31 per cent. In the two remaining experiments the changes in ventricular function curves were similar but not as consistent.

It would appear from the data shown in Figure 2 that further coronary vasodilation becomes increasingly difficult to achieve at low hematocrits. With this in view, depression of the ventricular function curve during anemia might

reasonably be interpreted as reflecting the inability of the coronary bed to dilate sufficiently to compensate for the decreased oxygen carrying capacity of the blood.

Effect on Myocardial Contractility of Artificially Augmenting Coronary Flow at Normal and Low

the ventricular function curve had been depressed by anemia (hematocrits of 17.5 and 24%), perfusion of the coronaries at a high rate appreciably improved myocardial contractility and returned the ventricular function toward normal.

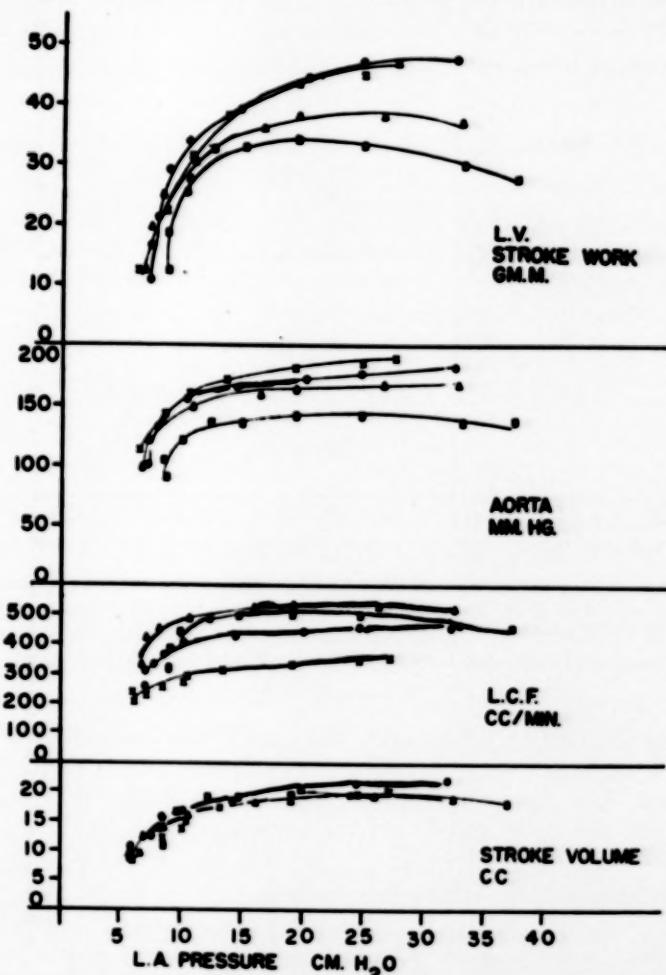


FIG. 4. Ventricular function curves in anemia. Experiment No. 88; dog weight 23.8 kg.; pericardium intact; vagotomy. At the top, the left ventricular work is plotted against mean left atrial pressure (ventricular function curves). Also shown are aortic pressure, left main coronary artery flow and stroke volume plotted against mean left atrial pressure. $\times \times \times$ = hematocrit of 43 per cent; 000 = 32 per cent; $\Delta \Delta \Delta$ = 24 per cent; $\square \square \square$ = 17.5 per cent. Note the depression of the ventricular function curve at 24 per cent and the further depression and descending limb at 17.5 per cent.

Hematocrits. During the course of three experiments left coronary artery flow was artificially augmented by means of a perfusion pump in parallel with the main coronary tubing. In the first of these, at a normal hematocrit, markedly increasing coronary flow did not alter the left ventricular stroke work per unit of filling pressure. Contrariwise in two experiments, after

Effects of Anemia in the Presence of Coronary Stenosis. A uniform coronary constriction was produced by partially clamping a by-pass in parallel with the main coronary tubing. Thereafter, when desired, coronary stenosis could be simulated by totally occluding the main coronary tubing, thus forcing all flow through the high resistance by-pass. Ventricular function curves

were obtained before and during application of this standard constriction at three different hematocrits. The results of this experiment are shown in Figure 5. At a hematocrit of 49.5 per cent (A) the constriction curve is seen to be depressed and to have a descending limb, as has

measured at varying left atrial pressures and work loads, and at each of two hematocrits. It appears that at any given hematocrit the coronary arteriovenous oxygen difference remains almost constant at varying work loads. Also, the removal of oxygen from the coronary

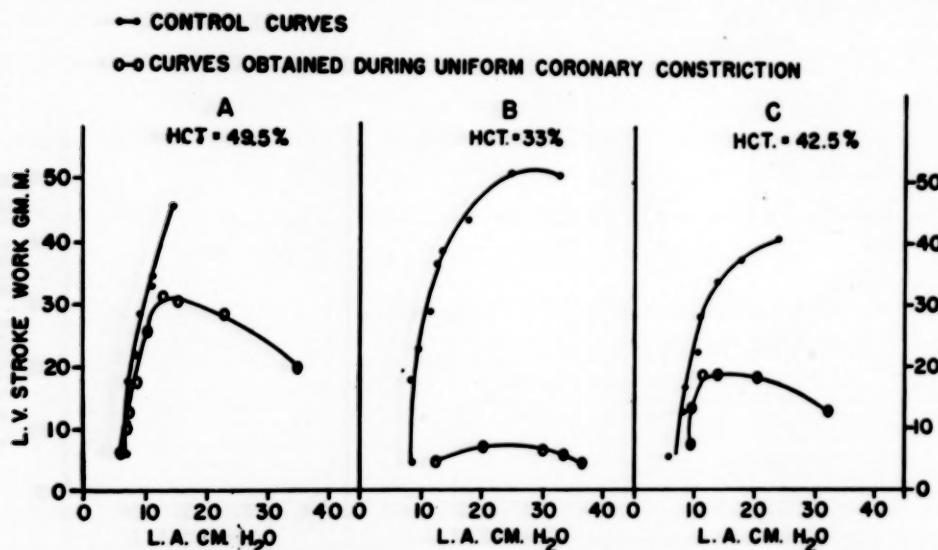


FIG. 5. Effect of coronary stenosis and anemia on ventricular function curves. Experiment No. 116; dog weight 21.2 kg.; pericardium intact; vagotomy. Dots represent points from control curves. Circles represent points obtained during application of a uniform coronary constriction. Dog was made anemic between A and B, and red cells were reinfused between B and C.

previously been demonstrated.⁵ When the hematocrit was reduced to 33 per cent (B), the constriction curve was even more severely depressed. A return of red cells raised the hematocrit to 42.5 per cent (C), resulting in a constriction curve intermediate in height between A and B.

It appears from the data shown in Figures 1 to 3 that dilation of the coronary vessels is an important facet of the circulatory compensation to anemia. In the presence of coronary stenosis, however, the effect of arteriolar dilatation in increasing coronary flow is minimized by the fixed high resistance of the stenotic artery. With coronary stenosis, therefore, myocardial depression occurs at lesser degrees of anemia. The degree of myocardial depression will, of course, be a function both of the degree of stenosis⁵ and the severity of the anemia.

Effect of Anemia on Coronary Sinus Oxygen Content and Arteriovenous Oxygen Difference at Various Work Loads. Table 1A shows data from two experiments in which the arterial and coronary sinus oxygen contents were simultaneously

blood is more complete at lower hematocrits, as also demonstrated by Bing et al. in three anemic patients.¹² Lastly, the decreased oxygen carrying capacity of the blood during anemia is largely compensated for by an increased coronary blood flow and, to a lesser extent, by more complete removal of oxygen (lower sinus content).

When an augmentation of blood flow is limited because of stenosis, increased removal of oxygen becomes a relatively more important compensatory mechanism and the coronary sinus content therefore falls. If, however, the possibility of increased oxygen removal is already limited because of the lowered coronary sinus content during anemia, this secondary type of compensation also becomes correspondingly less effective. (Table 1B.) The markedly depressed ventricular function curve that resulted from this combination of stenosis and anemia is shown in Figure 5B.

A further point of interest in these experiments (Table 1A) is that the coefficient of oxygen extraction, $\frac{A - V}{A}$, remains constant with vary-

Effect of Anemia on Coronary and Ventricular Function—Case et al. 403

ing filling pressures, work loads and, presumably, diastolic fiber lengths not only at any given hematocrit but also at varying hematocrits in the same dog. When, however, coronary insufficiency is present, (Table 1B) the coefficient of oxygen extraction becomes markedly elevated. It seems likely that this coefficient is basically an expression of the diffusion gradient of oxygen between the capillary blood and myocardial

tissue. For a more detailed consideration of myocardial coefficients of extraction the reader is referred to the writings of Goodale, Hackel and Kleinerman.^{13,14}

Changes in Peripheral and Pulmonary Resistances. The peripheral vascular resistance (excluding that of the coronary arteries) demonstrated a progressive fall as the hematocrit was reduced. The values obtained when plotted against the

TABLE I

Exp. No.	Hematocrit (%)	Mean Left Atrial Pressure (cm. H ₂ O)	Left Ventricular Work (kg.M./min.)	Arterial O ₂ Content (vol. %)	Coronary Sinus O ₂ Content (vol. %)	A-V O ₂ Difference (vol. %)	A-V/A	Left Coronary Flow (cc./min.)	Myocardial O ₂ Consumption (cc./min.)†
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A. Without Coronary Stenosis

107	38	10.9	2.97	16.5	5.4	11.1	0.67	148*	16.4*
	38	13.2	4.55	16.2	5.3	10.9	0.67	137	14.9
	38	15.4	6.46	15.9	5.2	10.7	0.67	162	17.4
	38	22.2	8.07	16.3	5.3	11.0	0.67	190	20.9
	38	35.3	8.92	16.9	5.9	11.0	0.65	208	22.9
	38	16.4	5.4	10.9	0.67 Average
	24	11.6	2.87	9.0	3.0	6.0	0.67	202	12.2
	24	14.7	5.30	9.7	4.1	5.6	0.58	282	15.8
	24	18.6	7.05	9.4	3.7	5.7	0.61	327	18.7
	24	34.2	7.43	9.4	3.1	6.3	0.67	337	21.2
	24	9.4	3.5	5.9	0.63 Average
116	49.5	8.7	4.65	19.1	5.1	14.0	0.73	118	16.5
	49.5	11.2	6.82	19.3	5.2	14.1	0.73	167	23.5
	49.5	14.4	9.13	19.1	5.2	13.9	0.73	230	31.8
	49.5	19.2	5.2	14.0	0.73 Average
	33	9.7	4.92	12.8	3.3	9.5	0.74	171	16.2
	33	12.6	7.47	13.1	3.4	9.7	0.74	254	24.7
	33	24.3	10.33	13.2	3.6	9.6	0.73	386	37.2
	33	13.0	3.4	9.6	0.74 Average

B. With Coronary Stenosis

116	49.5	7.0	2.28	18.8	4.4	14.4	0.77	60	8.6
	49.5	8.8	3.71	19.1	4.8	14.3	0.75	90	12.8
	49.5	22.9	5.64	19.1	2.8	16.3	0.85	133	21.7
	33	20.1	1.56	12.2	1.3	10.9	0.89	58	6.3
	33	36.8	0.99	12.7	1.2	11.5	0.91	41	4.7

* Questionable value.

† O₂ consumption of myocardium supplied by left main coronary artery.

pressure perfusing the peripheral bed formed a series of curves not unlike those illustrated for coronary resistances, but the changes were not as marked.

Pulmonary vascular resistance, when plotted against pulmonary artery pressure, showed slight and inconsistent changes during anemia.

Proportion of Cardiac Output Carried by the Coronary Vessels. The proportion of total cardiac output carried by the left coronary artery increased as the anemia progressed. For example, in the experiment illustrated in Figure 2 the left coronary artery flow was 2.9 per cent to 4.3 per cent of cardiac output at a hematocrit of 55 per cent. However, when the hematocrit was 19 per cent the left coronary flow was 11.0 to 12.4 per cent of the cardiac output. This threefold increase in the proportion of the cardiac output carried by the left coronary artery during anemia indicates that the increased coronary flow is not attributable to the lower blood viscosity. Rather it is due to a greater decrease in coronary vascular tone than that which occurs in the peripheral bed. It supports the view that the decreased coronary resistance occurs in response to the metabolic needs of the myocardium.

DISCUSSION

Because of the high extraction of oxygen from blood flowing through ventricular muscle, the main compensatory mechanism for decreased oxygen content of the blood is an augmentation of coronary flow resulting from a decreased resistance. The concept that the coronary arterioles maintain a precise balance between the myocardial oxygen need and availability has gained increasing support in recent years.^{15,16,20} In 1945 Shipley and Gregg concluded that the increase in coronary flow resulting from stellate ganglion stimulation was largely if not entirely a phenomenon secondary to the increased cardiac work which resulted from this stimulation.¹⁵ Eckenhoff and associates¹⁶ have supported and broadened this concept. They demonstrated that the coronary flow response resulting from changes in peripheral resistance, cardiac work and systemic flow was best related to changes in myocardial oxygen consumption. They also demonstrated that when the arterial oxygen content was reduced by low oxygen breathing the coronary vascular resistance decreased. This type of response has also been found following the release of induced coronary

stenosis or occlusion and intracoronary cyanide injections.^{5,17}

It would appear from the data herein presented that at normal hematocrit levels the maximal stroke work of the ventricles is not restricted by a limitation of coronary flow. In support of this is the observation that at maximal ventricular stroke works the coronary vascular resistance is still relatively high at normal hematocrits. Further, if, at maximal work rates, coronary flow is then artificially augmented by means of a perfusion pump no further increase in work occurs. It therefore seems probable that, with a normal arterial oxygen content and constant nervous and hormonal influences, the maximal ventricular work is determined by factors within the heart muscle itself.

The ability of the heart to meet the demands of the body in anemia depends to a large extent upon increased coronary flow. When the limits of dilatation of the coronary bed have been approached a higher ventricular filling pressure is necessary for a given amount of stroke work, i.e., a depressed ventricular function curve results. This phenomenon, shown in Figure 4, is similar to that observed when coronary artery flow is mechanically restricted.⁶ The cardiomegaly frequently seen in patients with severe chronic anemia may reasonably be explained on this basis, especially since heart size usually decreases when the anemia is alleviated.¹⁸

An increased work load of the heart requires an increased coronary flow. During anemia the augmentation of coronary flow required for any given work increase is even more pronounced. (Fig. 3.) Coronary stenosis limits this necessary flow increase, and the degree of work limitation imposed by the stenosis therefore will be compounded by the presence of anemia. (Fig. 5.)

The accentuation of angina pectoris by anemia is well known, and symptoms often improve when the hemoglobin is elevated.¹⁹ The data cited may furnish a basis for the more complete understanding of these phenomena.

CONCLUSIONS

1. Left main coronary artery flow, cardiac output, right and left atrial, and pulmonary artery and aortic pressures were continuously recorded in the open-chest, narcotized dog with a complete circulation. Ventricular function curves (modified Starling curves) were obtained in the control state and at varying degrees of anemia produced by the replacement

of blood with dextran. Calculations were made of coronary resistance and ventricular work.

2. A greater coronary flow per unit of left ventricular work occurs during anemia. The increment is a function of the severity of the anemia.

3. The decreased oxygen carrying capacity of the blood was largely compensated for by an increased coronary flow which resulted from lower coronary resistance. The resistance decreased progressively as the hematocrit was reduced and approached a minimal value at low hematocrit levels. More complete removal of oxygen from coronary blood was a limited but significant factor.

4. Depression of the ventricular function curve (less work per unit of filling pressure) did not occur until the hematocrit was reduced to between 24 and 31 per cent. After this, however, the curve was progressively depressed as the hematocrit was decreased. A descending limb appeared on the ventricular function curve when depression of the curve became marked.

5. The apparent cause of the depression of the ventricular function curve in severe anemia is that the coronary vessels have approached maximal dilatation and cannot further compensate for the decreased oxygen carrying capacity of the blood.

6. In the presence of coronary stenosis, anemia accentuated the depression of ventricular function.

7. The presence of a substantial vasodilatory reserve at high work loads with a normal hematocrit suggests that, under these circumstances, maximal ventricular stroke work is not limited by the availability of oxygen.

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The Use of Simultaneous Left Heart Pressure Pulse Measurements in Evaluating the Effects of Mitral Valve Surgery*

HOWARD L. MOSCOVITZ, M.D., ALVIN J. GORDON, M.D., EUGENE BRAUNWALD, M.D.,
SALOMAO S. AMRAM, M.D., SAMUEL O. SAPIN, M.D., RICHARD P. LASER, M.D.,
AARON HIMMELSTEIN, M.D. and MARK M. RAVITCH, M.D.

New York, New York

IT is the purpose of this report to discuss the application of a method for determining the hemodynamic effectiveness of mitral valvulotomy by obtaining direct pressures simultaneously from the left auricle and the left ventricle at the time of operation. This technic was developed to afford a physiologic means for early and objective evaluation of the success or failure of the operative procedure.

At present, the clinical response of the patient and postoperative cardiac catheterization are the two major determinants of the adequacy of mitral valvulotomy. Both methods have certain shortcomings. The subjective response of the patient may be misleading in assessment of the degree of relief of mechanical obstruction that follows mitral valvulotomy. In some patients consciousness of heart disease has become so deep-seated that even after apparently adequate valvulotomy has been performed no symptomatic improvement takes place. Conversely, significant subjective improvement has been claimed by some patients who have had nothing more than a fruitless thoracotomy during which no definitive mitral valvulotomy could be performed because of some technical difficulty¹ or because of the presence of marked mitral insufficiency.² We have observed this several times. An instructive case illustrating the difficulty that may be encountered in evaluating the effect of commissurotomy on the basis of the clinical response of the patient follows:

F. S., a forty-six year old white housewife, had rheumatic polyarthritis at the age of ten. Congestive heart failure developed during both of her pregnancies, at twenty-seven and thirty-eight years of age, and she was maintained on

adequate digitalis therapy since the second episode of failure. She had been in continuous congestive failure for the eight years prior to surgery, with severe, progressive dyspnea and three-pillow orthopnea. She required injections of mercuhydrin three times weekly to forestall a rapid gain in weight. This history, together with the findings of pure mitral stenosis on physical examination, constituted the indication for mitral valvulotomy. At thoracotomy the left auricle and its appendage were found to be almost completely filled with organized thrombus, which was adherent to the auricular wall. The surgeon was unable to approach the mitral valve. Direct pressure measurements, by the method to be described, revealed the mean left auricular pressure to be elevated to 29 mm. Hg, with a mean filling pressure gradient across the mitral valve of 18 mm. Hg, indicating hemodynamically significant mitral stenosis. The chest was closed without any attempt to open the left auricle. When the patient was seen at follow-up four months after operation, she declared herself to be markedly improved. She could climb stairs slowly, slept flat in bed and was able to do some housework for the first time in years. The patient had received no diuretics in the preceding five weeks.

Disappearance of murmurs, diminution in cardiomegaly and regression of electrocardiographic abnormalities are too inconstant to be conclusive criteria of the success of the valvulotomy.

The expectation that follow-up cardiac catheterization would accurately and objectively correlate the hemodynamic changes that follow mitral valvulotomy with the subjective response of the patient has not uniformly been borne out.

* From the Departments of Medicine and Surgery of the Mount Sinai Hospital, New York, N. Y.

Good correspondence between the drop in pressures in the pulmonary circuit and the clinical improvement of the patient has been reported in several series of cases studied postoperatively.³⁻⁵ However, a similar degree of subjective improvement has been noted in a significant number of patients despite the fact that this was not reflected by the pulmonary artery pressure, pulmonary arteriolar resistance and cardiac output, as determined by cardiac catheterization months after the mitral valvulotomy has been performed.^{1,6-10} Jordan and Hellems⁸ remark, "That all these patients have been improved clinically is undeniable, but on examination of the pulmonary artery and pulmonary capillary pressures, one is not so confident of the results."

There are several reasons why postoperative cardiac catheterization may not fully reflect the hemodynamic changes produced by mitral valvulotomy. It is possible, as pointed out by several workers,^{4,11,12,14} that the results obtained when postoperative catheterization is performed soon after operation may not reveal the entire change that will occur after longer periods have elapsed. It must be kept in mind that right heart catheterization can describe what is taking place at the mitral valve only indirectly, by recording the effect of the mitral disease on the pulmonary circuit. In some patients with high and relatively fixed pulmonary arteriolar resistance the changes that have occurred in the pulmonary vascular bed may be irreversible or only slowly regressive. In such patients, when the pulmonary artery mean pressure exceeds 60 mm. Hg, it has been reported that pulmonary artery wedge (pulmonary capillary) pressures may be difficult to obtain.^{6,15} Under these circumstances it would be impossible to calculate the alteration in pulmonary arteriolar resistance and in left auricular pressure that followed commissurotomy. Undoubtedly, in many of these patients generous valve orifices have been created by the surgeon. Persistence of high pulmonary artery pressures and failure of the cardiac output to rise postoperatively cannot be taken as conclusive evidence that an inadequate valvulotomy has been performed.

Persistence of an elevated pulmonary capillary pressure does not necessarily indicate that the mitral stenosis has not been relieved since this pressure may be high in conditions other than mitral stenosis. It is elevated both in mitral

stenosis and in left ventricular failure, and the two cannot be differentiated by the venous cardiac catheter. Left ventricular failure may follow mitral surgery because of inadvertent production of mitral insufficiency, because of the progression of aortic valve disease, or as a result of active myocarditis. On the other hand, a fall in pulmonary capillary pressure may not always indicate a beneficial result of mitral surgery. It is conceivable that if mitral insufficiency is accidentally produced at the same time that the stenosis is relieved, the left auricular pressure may fall moderately, while the left ventricular diastolic pressure may rise considerably. In such instances postoperative cardiac catheterization would reveal only a somewhat lower pulmonary capillary pressure and give no evidence of the rise in left ventricular diastolic pressure. Finally, changes in medical therapy, the removal of precipitating causes of heart failure and the occurrence of fluctuations in the level of activity of the rheumatic state must all be considered in interpreting the findings of postoperative cardiac catheterization.¹²

The need for an objective test to determine the effectiveness of mitral valvulotomy is apparent. The problem is to establish how much mitral stenosis remains after surgery. Measurement of the filling pressure gradient across the mitral valve is the only direct method of determining the physiologic degree of mitral stenosis. Although the importance of this left atrioventricular pressure gradient has been mentioned by some authors,^{16,17} it has not been analyzed in detail chiefly because direct graphic representation has not previously been accomplished in man. Measurements of the left auricular and left ventricular pressures in the human heart have been made by a number of workers, using a variety of methods,¹⁸⁻²⁶ but synchronous left auricular and left ventricular pressures, recorded on curves of identical sensitivity, have not thus far been presented. We have made simultaneous pressure recordings in cases of mitral stenosis before and after valvulotomy by inserting needles directly into the left auricle and the left ventricle at the operating table.^{27,28} By inscribing these pressures synchronously, using manometric systems adjusted to equal sensitivity, an accurate measurement of the mitral valvular filling pressure gradient has been obtained. Additional studies have been made in control patients with normal mitral valves.



FIG. 1. Simultaneous left auricular, left ventricular and aortic pressures in a normal control subject. Note the absence of a pressure gradient between the left auricle and left ventricle during diastole.

METHOD

A detailed description of the method appears elsewhere.²⁸ Three Statham pressure transducers (P 23 A) are set up in a bank on a single stand. Identical zero points are set for each gauge on the monitoring screen of a four-channel oscillographic recorder. A known pressure from a large pressure reservoir connected to a mercury manometer is then applied to each of the strain gauges. The sensitivity of all three pressure channels is then adjusted so that the applied pressure from the reservoir produces equal deflections on each channel. The system is now ready to record, synchronously, triple pressures at identical sensitivity from identical zero points. Each of the three-strain gauges is connected to a 4-foot length of autoclaved vinyl tubing. The three tubes are attached to No. 20 needles which are inserted by the surgeon into the left auricle, the left ventricle and the aorta, respectively, in the open chest. The three pressure pulses are inscribed simultaneously, together with an electrocardiographic lead. This method provides an opportunity to record accurately the pressure pulse of each chamber in relation to that of other chambers of the left heart, without the necessity for tracing, transferring or artificial superimposition, processes which are laborious and may be inaccurate. The diastolic pressure gradient across the mitral valve may be estimated visually at operation from the oscilloscopic screen, and a photographic record is obtained for careful measurement and timing of the hemodynamic events of the left heart.²⁸ This method of direct puncture of the cardiac chambers and

great vessels has been free of complications in our experience thus far.

RESULTS

Normal Mitral Filling Pressure Gradient. The differential pressure between the left auricle and the left ventricle during the time that the mitral valve is open will be designated "the mitral filling pressure gradient." The term "filling pressure gradient" is used rather than "diastolic pressure gradient" since it appears from our observations²⁸ that in mitral stenosis filling of the left ventricle may take place during a small portion of systole.

Simultaneous recordings of the pressure pulses on the left side of the heart in six control cases with no clinically detectable mitral valve disease have been obtained. These pressures were recorded during thoracotomy for removal of a thymic cyst, resection of carcinoma of the lung or tuberculoma, and after ligation of a patent ductus arteriosus. Virtually no gradient of pressure between the left auricle and the left ventricle was found throughout the diastolic period in such instances. (Fig. 1.) This is true even during the period of greatest mitral flow, which occurs immediately after the opening of the mitral valve.²⁹ Some slight pressure gradient must exist for blood to flow across even the normal mitral valve. However, it appears that the pressure differential responsible for such a large flow is barely detectable.

The Gradient in Mitral Stenosis before and after Valvulotomy. Representative curves of the left heart pressure pulses before and after valvulotomy in patients with mitral stenosis are shown in Figures 2 to 5. A variable pattern will be noted if attention is directed to the filling pressure gradient between the left auricle and the left ventricle.

Figure 2 illustrates the pressure curves obtained before and after valvulotomy in a patient (E. H.) with mitral stenosis in whom the mitral orifice was surgically enlarged from an aperture that would not admit a fingertip to one that was estimated to accommodate two fingers. The mean filling pressure gradient across the mitral valve, 19 mm. Hg before valvulotomy, was reduced to 3 mm. Hg after valvulotomy. This reduction in gradient was accomplished solely by a fall in the left auricular pressure; there was no rise in the left ventricular diastolic pressure. This represents an excellent hemodynamic result and the valvulotomy can be classified as adequate.

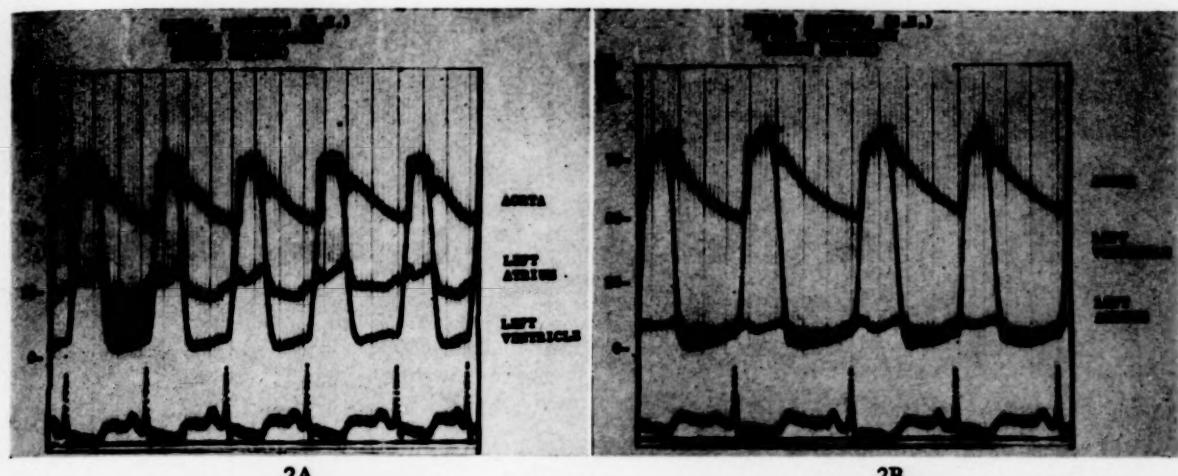


FIG. 2. A and B, simultaneous left auricular, left ventricular and aortic pressures in a patient with mitral stenosis. The filling pressure gradient (shaded in the first beat) has fallen markedly following valvulotomy.

In patient E. S. (Table 1) an elevated mean filling pressure gradient between the left auricle and the left ventricle, 11 mm. Hg, was reduced to zero. This occurred due to a simultaneous fall in the mean left auricular diastolic pressure, from 23 to 16 mm. Hg, and a rise in the mean left

ventricular diastolic pressure, from 12 to 16 mm. Hg. The surgeon estimated that the postoperative mitral orifice would accommodate three fingers. No regurgitant jet was produced. Restoration of the mitral filling pressure gradient to zero without concomitant production of

TABLE I
SUMMARY OF ABSOLUTE PRESSURES OBTAINED IN THE LEFT HEART OF SEVEN PATIENTS BEFORE AND AFTER
MITRAL VALVULOTOMY
(mm. Hg)

Patient	Left Auricular Pressure		Mitral Valve Filling Pressure Gradient	Left Ventricular Pressure (systolic/diastolic)	Aortic Pressure (systolic/diastolic)
	Mean	Diastolic Mean			
I. M. Preoperative.....	26	24	14	94/12	95/58
Postoperative.....	21	19	8	95/12	99/65
S. W. Preoperative.....	24	23	20	84/3	85/51
Postoperative.....	26	19	14	92/5-9	96/61
S. K. Preoperative.....	11	8	4	65/4	65/44
Postoperative.....	11	7	1	86/6	86/52
S. P. Preoperative.....	28	27	17	91/10	87/60
Postoperative.....	18	15	8	95/7	95/50
E. S. Preoperative.....	24	23	11	85/12	83/58
Postoperative.....	20	16	0	108/16	108/71
E. H. Preoperative.....	29	28	19	76/9	76/53
Postoperative.....	9	7	3	84/4	85/53
M. G. Preoperative.....	13	12	5	91/7
Postoperative.....	7	6	0	92/6	92/52

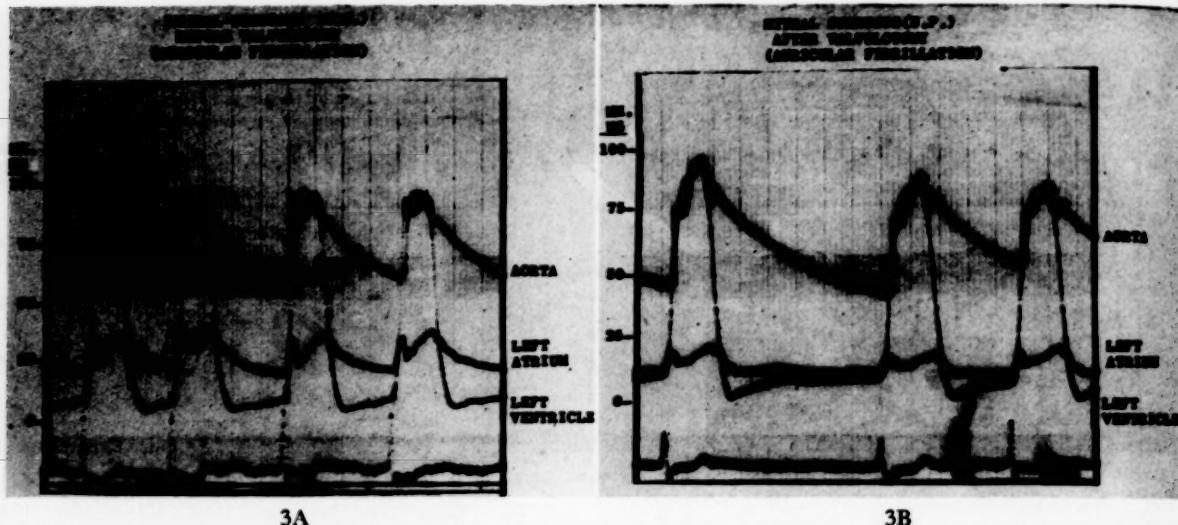


FIG. 3. A and B, simultaneous left auricular, left ventricular and aortic pressures in a patient with mitral stenosis. Note the influence of the length of diastole on the magnitude of the left atrioventricular filling pressure gradient in the post-operative tracing.

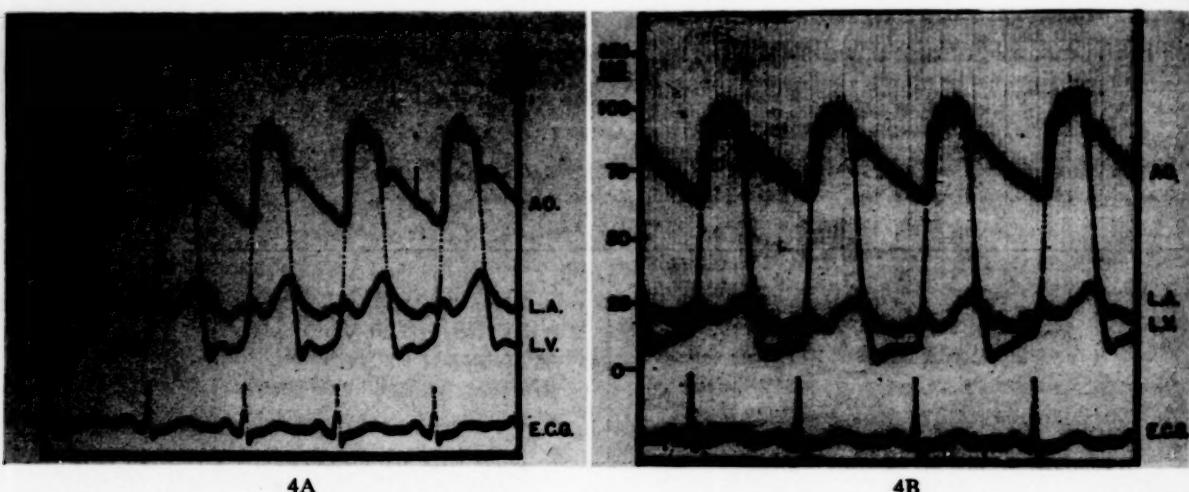


FIG. 4. (I. M.) A, simultaneous left auricular, left ventricular and aortic pressures in a patient with mitral stenosis before valvulotomy. B, note that the filling pressure gradient has been reduced only partially following valvulotomy.

mitral insufficiency can also be classified as a very satisfactory valvulotomy.

Figures 3 and 4 present examples of patients in whom only partial reduction of the left atrioventricular pressure gradient occurred following commissurotomy. In both cases an elevated mean filling pressure gradient of 17 mm. Hg (S. P.) and 14 mm. Hg (I. M.), respectively, was reduced to 8 mm. Hg without elevation of the left ventricular diastolic pressure. At no point during the diastolic period, however, did the gradient in either case approximate the normal and some residual mitral stenosis in all likelihood remains. Despite this, in both cases the

mitral orifice was felt to have been opened widely enough to accommodate at least two fingers.

In Figure 5 an example of the result of an unsatisfactory valvulotomy is shown. The mean left auricular diastolic pressure fell from 23 mm. to 19 mm. Hg, while the mean left auricular pressure actually rose from 24 to 26 mm. Hg. This rise in mean left auricular pressure occurred despite reduction of the mitral stenosis from the size of a fingertip to that of one and one-half fingers. The rise in left auricular pressure in this instance is due primarily to production of a high late-systolic peak in the left auricular curve, undoubtedly representing surgi-

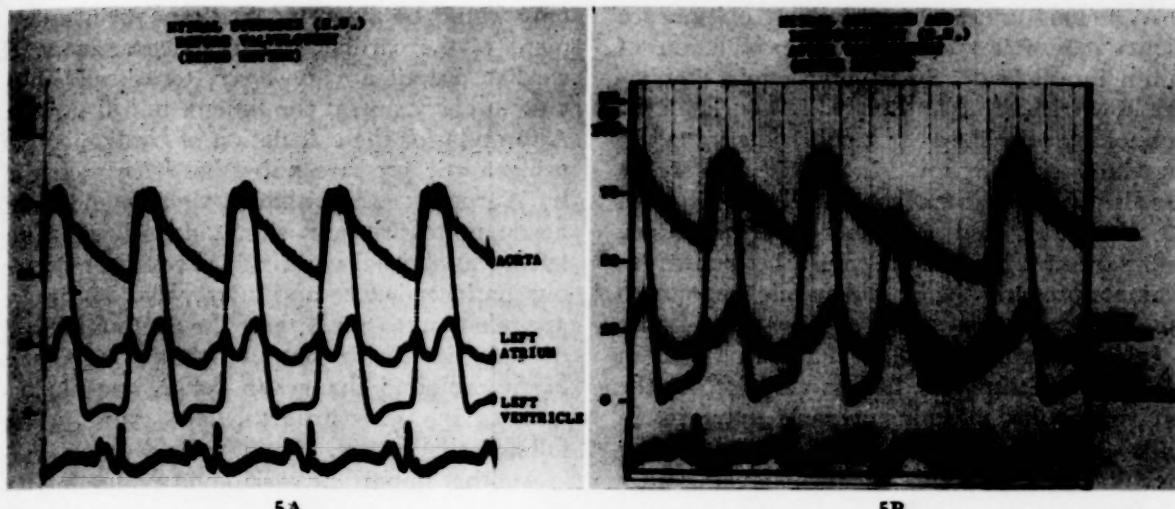


FIG. 5. A and B, simultaneous left auricular, left ventricular and aortic pressures in a patient with mitral stenosis. Note the appearance of a high late-systolic peak in the postvalvotomy auricular tracing following the inadvertent production of mitral insufficiency.

cally produced mitral insufficiency. A regurgitant jet of moderate volume was felt by the finger in the atrium after commissurotomy. In addition, a distinct rise in the left ventricular end-diastolic pressure has taken place and a slight rise in its mean pressure. The combination of a slight fall in the mean left auricular diastolic pressure and a slight rise in the mean left ventricular diastolic pressure reduced the filling pressure gradient across the mitral valve from 20 to 14 mm. Hg. Despite this fall in gradient, however, the hemodynamic relationships are less satisfactory than before operation, because of mitral insufficiency, and this has been borne out by the unfavorable postoperative clinical course of the patient.

A summary of the absolute pressures as determined in a group of seven patients with mitral stenosis, studied in the manner described, is presented in Table I.

COMMENT

The control tracings obtained in human subjects with uninvolved mitral valves show that mitral valve filling pressure gradient normally is very small. In the presence of a grossly deformed and calcified valve, with a tiny or eccentrically placed orifice, one might expect that in a certain percentage of cases complete physiologic reduction of the stenosis might be impossible. The surgeon's estimate of the size of the anatomic opening of the valve which he has produced may be in error. It is conceivable

that with a pliable and rubbery valve the commissures may be partially split and then further dilated by the surgeon's finger, only to resume a significantly smaller diameter when the finger is withdrawn. This may have been the case in patients I. M. and S. P. in whom the pressure gradient was only partially reduced although the surgeon felt that an anatomically adequate orifice had been produced.

It may be too much to require that in every case the diastolic pressure gradient across the mitral valve approach the normal after valvotomy, since in many cases the valve function will have been severely deranged by shrinkage of the leaflets or involvement of the subvalvular structures. This, however, does not detract from the usefulness of the test in evaluating the extent of the valvotomy and the degree of residual stenosis.

It is apparent that the left atrioventricular filling pressure gradient may be altered following valvotomy in a manner quite independent of the direction and degree of change of the left auricular pressure. The gradient may fall due solely to a drop in the left atrial pressure. (Fig. 1, E. H.) The gradient may be reduced by a rise in the left ventricular diastolic pressure with or without a concomitant fall in the left atrial pressure. (Table I, E. S.) It is possible, in fact, that the gradient may be reduced despite an increase in the left atrial pressure, if the left ventricular diastolic pressure develops a greater rise than does the left atrial pressure. (Fig. 5,

S. W.) This unusual combination of pressure events may follow inadvertent production of mitral insufficiency. Such observations emphasize how misleading an isolated left auricular or pulmonary capillary pressure determination may be in assessing the degree to which surgery has altered the degree of the mitral stenosis. Both the left atrial and left ventricular pressures, simultaneously registered, must be known to evaluate properly the hemodynamic response to mitral valvulotomy.

It has been our experience that with the fall in left atrial pressure that follows valvulotomy the left ventricular diastolic pressure may rise in some cases, further reducing the mitral filling pressure gradient. This rise in left ventricular diastolic pressure may be a function of increased mitral flow; in some cases it is a transient occurrence likely to disappear as the left ventricle adjusts itself to the increased load.

Mitral valvular flow and the alterations in this flow that follow valvulotomy must be taken into account in assessing the change in pressure gradient that occurs after the surgical procedure. Since the pressure relationships depend both on mitral valvular resistance and on flow, it is conceivable that with the increase in flow that may follow partial relief of the mitral stenosis the left atrioventricular diastolic pressure gradient may not fall markedly. This is especially true since flow across the stenotic mitral valve is turbulent and small increases in flow lead to considerable increases in pressure. No data are available concerning any possible change in the mitral diastolic pressure gradient that may occur in the normal heart when the flow increases. However, since pulmonary capillary pressures normally show at most a minimal rise during exercise,^{6,20} when the cardiac output increases markedly, it may be inferred that an adequate mitral valve can accommodate large increments in flow with no significant rise in the pressure gradient across the valve. Thus it is logical to assume that if the mitral filling pressure gradient remains high because of the increased flow that follows valvulotomy, this must mean that significant residual stenosis remains. It is unlikely that under the conditions of the operation the mitral valvular flow will increase so markedly as to maintain a high filling pressure gradient across the mitral valve if the commissurotomy has been completely adequate.

There is no doubt that proper assessment of the change in filling pressure gradient after valvulot-

omy would be aided by simultaneous measurement of the cardiac output. Measurements of cardiac output are, however, technically difficult to carry out at the time of mitral surgery. A formula for the calculation of cardiac output using aortic pressure pulses has been presented by Warner et al.²¹ Although we have measured aortic pulse pressures simultaneously with the left atrial and left ventricular pressure pulses in our patients before and after valvulotomy, we are reluctant to apply the pulse contour method of estimating cardiac output to these cases because of the profound changes in the physical characteristics of the cardiovascular system which follow valvulotomy.

Another important variable in evaluating the mean filling pressure gradient in mitral stenosis is the length of the diastolic period. Prolonged diastole allows the left auricle to empty itself more effectively (Fig. 3), and at very slow rates even high degrees of mitral stenosis may not be characterized by excessive gradients throughout the diastolic period. It can easily be appreciated that tachycardia imposes a further burden on a heart with mitral stenosis by reducing the time for left auricular emptying and therefore for left ventricular filling.

Measurement of the left atrioventricular filling pressure gradient would appear to have several other advantages. It affords an accurate method of diagnosing mitral stenosis in the living and unopened heart. It differentiates mitral stenosis from left ventricular failure, pulmonary vein thrombosis, constrictive pericarditis and cor triatriatum, conditions which produce elevated left atrial pressure or elevated pulmonary capillary pressure.^{17,22,23} The method provides an instantaneous visual picture to the surgeon at the operating table of the gradient across the mitral valve, thereby assisting him in determining whether he has sufficiently split the valve or whether further manipulative procedures are necessary. Recording of pressure gradients across stenotic valves at the time of operation have greatly aided the surgical attack on congenital pulmonic stenosis^{22,24} and should prove to be of use in the surgery of aortic stenosis.

The method may have application in those patients previously operated upon for mitral stenosis who are being considered for a second valvulotomy because of return of symptoms. Recurrences of stenosis do occur.^{8,25,26} However, it is extremely difficult, short of re-exploration of the valve, to determine whether valvular

stenosis has recurred or the return of symptoms is due to myocardial failure or superimposed valvular lesions. After removal of the auricular appendage at the first operation, re-entry into the left auricle may be exceedingly difficult and dangerous. Therefore, the pressure in the left auricle and left ventricle could be measured at the second thoracotomy, and if an elevated diastolic pressure gradient was not found re-entry into the left auricle and a second attempt at valvulotomy would be contraindicated. If the pressure gradient could be recorded safely and accurately by closed chest technic¹⁸⁻²⁰ these methods would have obvious advantages in such cases.

SUMMARY

1. The need for an objective method of measuring the adequacy of mitral valvulotomy by estimating the extent of residual mitral stenosis is emphasized.

2. Measurement of the mitral valve filling pressure gradient appears to be the best method of determining the degree of hemodynamically significant mitral stenosis. A method for recording the pressure gradient between the left auricle and the left ventricle at the operating table, by inscribing simultaneous pressure pulses in the left heart, is described.

3. The normal filling pressure gradient across the mitral valve approximates zero. The effect of valvulotomy on the elevated pressure gradient in mitral stenosis is to produce a fall of variable degree, depending on the adequacy of the surgical procedure. When this pressure gradient remains high after valvulotomy, relief of the obstruction can be termed inadequate.

4. The method is also of value in the differential diagnosis of mitral stenosis, in aiding the surgeon at the operating table to decide whether sufficient manipulation of the valve has been carried out and in determining whether recurrence of obstruction has taken place in patients previously operated upon for mitral stenosis.

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The Precordial Electrocardiogram in Mitral Regurgitation*

ROLLAND D. BATEMAN, JR., M.D. and L. E. JANUARY, M.D.

Iowa City, Iowa

THE presence or absence of mitral regurgitation of sufficient degree to preclude operation on a stenotic valve is a frequent and important question which arises in the consideration of patients for mitral valvuloplasty. To resolve this question the presence of enlargement of the left ventricle is diligently sought. In most instances in which enlargement is great, little difficulty is encountered for clinical examination, supplemented by roentgenographic studies including cardiac fluoroscopy, promptly settles the question. In cases, however, which present minor degrees of left ventricular hypertrophy, often with a systolic murmur of less than Grade III intensity, current diagnostic technics often are inadequate. As a result many patients are subjected to thoracotomy or cardiotomy which cannot benefit them; indeed it may have a distinctly detrimental effect.

The present study was undertaken in an attempt to determine whether there are differences in the precordial electrocardiogram of patients with significant mitral regurgitation as compared with those having relatively "pure" mitral stenosis.

MATERIAL

In this hospital ninety-two patients have had thoracotomy for the surgical relief of mitral stenosis. At the time of operation nine (9.6 per cent) of these patients were found to have significant mitral regurgitation, despite pre-operative efforts to exclude patients with such contraindication to valvuloplasty.

This study deals with fifty-two patients with "pure" mitral stenosis followed six or more months after valvuloplasty and nine patients with significant mitral regurgitation.

ELECTROCARDIOGRAPHIC DIAGNOSIS OF LEFT VENTRICULAR HYPERTROPHY

The changes produced in the ECG by hypertrophy of the left ventricle are well known and

have been described in detail by Wilson et al.,¹ Barker,² Sokolow and Lyon,³ and Myers.⁴ They include slight prolongation of the QRS time, tall R waves with late onset of the intrinsicoid deflection (delayed ventricular activation time), commonly a prominent Q wave, downward displacement of the RS-T junction with an upwardly convex RS-T segment and an inverted T wave with its apex near the end, in leads reflecting the potential changes of the left ventricle. When these changes are found no difficulty is encountered in the electrocardiographic diagnosis of left ventricular hypertrophy. There are many patients with less left ventricular hypertrophy, however, who have some but not all of these changes. They were particularly numerous in this group. We assume that the development of the "typical" electrocardiographic pattern of left ventricular hypertrophy is gradual.⁵ Prior to massive hypertrophy, with the accompanying changes in muscle metabolism and the alterations in depolarization and repolarization, only minor changes in the precordial electrocardiogram are expected.

In this study changes in the voltages of the QRS complex and the time of onset of the intrinsicoid deflection in leads reflecting the potential variations of the left ventricle were measured and compared in the groups noted subsequently. Measuring of voltages in the precordial leads presupposes accurate standardization of the electrocardiograph. It is our practice to standardize each individual lead separately.

NORMAL VALUES

Reliable indices of the normal amplitude of QRS complexes in the precordial leads are difficult to define because the size of the deflection varies with the proximity of the electrode to the heart and the electrical conductivity of the tissues interposed between them. The shape of the chest, the amount of lung tissue overlying the heart, the thickness of the layer of sub-

* From the Cardiovascular Laboratory, Department of Internal Medicine, College of Medicine, State University of Iowa, Iowa City, Iowa.

cutaneous fat, edema of the subcutaneous tissues and edema of the lung are among the factors which influence the size of the QRS complex in precordial leads. Variations in these phenomena should occur with about equal frequency in each group of patients and thus be without serious influence on the observations.

Kossmann⁶ recently summarized the present knowledge of the normal standards for the electrocardiogram and listed the following, which we have used as the basis for comparison.

A. Voltages of QRS complexes in precordial leads in normal adults age twenty and over:

Deflection	No. of Cases	Min.	Max.	Mean
S V ₁	567	0.8	26.2	9.4
R V ₅	567	0.4	33.6	12.0

Mean sum of S V₁ and R V₅ is 21.4 mm.

B. Measurement in seconds of precordial electrocardiographic intrinsicoid deflections in normal subjects (lead V₅):

Group	No. of Cases	Min.	Max.	Mean
Kossmann and Johnston	30	0.023	0.053	0.0336
Sodi-Pallares	100	0.024	0.050	0.0370

In the groups which follow we measured the amplitude of the S wave in V₁ and of the tallest R wave in V₅ or V₆ for each patient. The sum represents the potential difference related to left ventricular depolarization. We then calculated the mean values for each of the deflections and for the sum of the two for each group. In addition, we measured the time of onset of the intrinsicoid deflection in V₅ or V₆, whichever was greater, and the mean value for each group was established.

RESULTS

Mitral Regurgitation. The results obtained in the nine patients with significant mitral regurgitation are summarized in Table I. The mean value of the time of onset of the intrinsicoid deflection (0.047 seconds) in V₅ or V₆ is distinctly above normal. Also, the mean value of the amplitude of the S waves in V₁, R waves in

V₅ or V₆ and the sum of the two (31 mm.) is well above the normal values. The "typical" pattern of left ventricular hypertrophy occurred in only two of these patients.

"Pure" Mitral Stenosis. Excellent clinical result. The results obtained in this group of twenty-one

TABLE I
MITRAL REGURGITATION (NINE CASES)

Patient No.	VAT V ₅ or V ₆ *	S V ₁	R V ₅	R V ₅ + S V ₁
1	.04	17	16	33
2	.05	4	14	18
3	.06	9	27	36
4	.06	5	26	31
5	.04	19	32	51
6	.04	5	16	21
7	.03	2	14	16
8	.05	7	16	23
9	.05	18	25	43
Mean	.047	9	21	30

* VAT = Ventricular Activation Time (time of onset of intrinsicoid deflection)

TABLE II
PATIENTS WITH "PURE" MITRAL STENOSIS WITH AN "EXCELLENT" RESULT (TWENTY-ONE CASES)

Patient No.	VAT V ₅ or V ₆ *	S V ₁	R V ₅	R V ₅ + S V ₁
12	.04	12	23	35
16	.05	11	12	23
22	.04	11	13	24
25	.03	7	7	14
27	.03	4	19	23
28	.03	13	14	27
30	.04	0	13	13
35	.03	0	11	11
38	.04	0	14	14
40	.04	0	22	22
41	.03	15	11	26
52	.06	6	13	19
53	.05	5	19	24
54	.03	0	7	7
55	.04	18	12	30
56	.05	10	28	38
57	.04	0	7	7
58	.03	17	24	41
65	.03	22	5	27
76	.02	0	6	6
84	.04	9	24	33
Mean	.038	8	14	22

* VAT = Ventricular Activation Time (time of onset of intrinsicoid deflection).

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patients are summarized in Table II. The mean value of the time of onset of the intrinsicoid deflection in this group was 0.038 seconds, essentially the same as the normal value. The mean values for the amplitude of the S wave in V₁, the R wave in V₅ or V₆ and the sum of these

insufficient for the results to be statistically significant.

Patients worse following valvuloplasty. The two cases were too few for the results to have statistical validity (Table V). The only value which varied from the normal was the mean of the

TABLE III
PATIENTS WITH "PURE" MITRAL STENOSIS WHO WERE CLINICALLY "IMPROVED" (TWENTY-FOUR CASES)

Patient No.	VAT V ₅ or V ₆ *	S V ₁	R V ₅	R V ₆ + S V ₁
13	.03	11	6	17
15	.02	2	6	8
17	.04	7	19	26
18	.02	0	11	11
19	.04	6	21	27
21	.04	4	6	10
24	.05	1	7	8
26	.03	2	8	10
33	.05	6	17	23
34	.05	7	21	28
37	.04	1	11	12
39	.03	18	22	40
42	.04	10	15	25
43	.04	5	29	34
44	.03	7	12	19
45	.04	15	12	27
50	.03	3	4	7
51	.03	8	12	20
60	.04	5	10	15
64	.04	13	12	25
67	.04	3	9	12
69	.03	6	18	24
71	.03	2	12	14
86	.04	14	13	27
Mean	.04	6	13	19

* VAT = Ventricular Activation Time (time of onset of intrinsicoid deflection).

deflections (22 mm.) are essentially normal values.

Patients "improved" following valvuloplasty. The results in the improved group of twenty-four patients are tabulated in Table III. In this group the mean value for the time of onset of the intrinsicoid deflection was very slightly above the normal value. The mean values for the amplitudes of the S wave in V₁ and the R wave in V₅ or V₆ and the sum of these deflections were normal.

Patients unimproved following valvuloplasty. The results in the unimproved group of five cases are tabulated in Table IV. The values obtained were not definitely different from those in the preceding two groups and the number of patients is

TABLE IV
PATIENTS WITH MITRAL STENOSIS WHO WERE UNIMPROVED (FIVE CASES)

Patient No.	VAT V ₅ or V ₆ *	S V ₁	R V ₅	R V ₆ + S V ₁
9	.03	2	7	9
23	.04	15	18	33
29	.04	7	6	13
36	.05	11	17	28
59	.04	1	21	22
Mean	.04	7	14	21

* VAT = Ventricular Activation Time (time of onset of intrinsicoid deflection).

TABLE V
PATIENTS WHO WERE CLINICALLY "WORSE" POSTOPERATIVELY (TWO CASES)

Patient No.	VAT V ₅ or V ₆ *	S V ₁	R V ₅	R V ₆ + S V ₁
11	.03	17	23	40
23	.04	2	10	12
Mean	.035	9	17	26

* VAT = Ventricular Activation Time (time of onset of intrinsicoid deflection).

sum of the S waves in V₁ and the R waves in V₅ or V₆. This value was slightly elevated.

COMMENTS

Table VI summarizes the results of this study. It will be noted that the nine patients in this series who had a degree of mitral regurgitation sufficient to preclude valvuloplasty had voltages of S waves in V₁ and R waves in V₅ or V₆ and a time of onset of the intrinsicoid deflection in V₅ or V₆ which were distinctly greater than the normal mean values. Furthermore, these values were definitely in excess of those in patients with "pure" mitral stenosis who had an excellent or improved operative result. This suggests that a carefully interpreted precordial electrocardiogram, combined with a thorough history and physical examination and careful roentgeno-

graphic and fluoroscopic examinations, can be of distinct value in detecting minor degrees of left ventricular hypertrophy such as are likely to be encountered in the group of cases under consideration. It may be extremely difficult to decide preoperatively whether dynamically

TABLE VI
SUMMARY (MEAN VALUES)

Classification of Cases	No. of Cases	S Wave in V ₁ (mm.)	R Wave in V ₅ or V ₆ (mm.)	S V ₁ + R V ₅ or V ₆ (mm.)	Time of Onset of Intrinsicoid Deflection in V ₅ or V ₆ (sec.)
Normal ^a	567	9.4	12	21
Normal ^b	100	0.0336
Normal ^c	30	0.0370
Mitral regurgitation.....	9	9	21	30	0.047
Mitral stenosis.....					
Excellent.....	21	8	14	22	0.038
Improved.....	24	6	13	19	0.040
Unimproved.....	4	7	14	21	0.040
Worse.....	2	9	17	26	0.035

significant mitral regurgitation exists. The best evidence of functionally significant regurgitation is enlargement of the left ventricle. Our data indicate that the precordial electrocardiogram is as valuable as most of the methods presently available, and more valuable than some, in detecting minor degrees of left ventricular hypertrophy, keeping in mind the extracardiac factors which have a pronounced influence on the size of the QRS deflection in precordial leads.

We do not propose relaxation of the present criteria for the electrocardiographic diagnosis of left ventricular hypertrophy in routine practice. The harm done to patients by overemphasis of minor electrocardiographic abnormalities is well known. In the highly specialized clinical situation dealt with here, however, the results were significant and might provide diagnostic help in a difficult situation.

SUMMARY

1. The precordial electrocardiograms of sixty-one patients with disease of the mitral valve are reviewed.

2. The time of onset of the intrinsicoid deflection in V₅ or V₆ was measured in each and mean values were obtained for each group of patients: those estimated at operation to have sufficient mitral regurgitation to contraindicate valvoplasty and those with "pure" mitral stenosis who had a valvoplasty and were later classed as excellent, improved, unimproved and worse from a functional standpoint.

3. The mean values of the amplitude of the S wave in V₁, the R wave in V₅ or V₆ and the sum of these values were obtained.

4. The group of patients who had significant mitral regurgitation had a distinctly later onset of the intrinsicoid deflection and greater voltages of S waves in V₁ and R waves in V₅ or V₆ than did the patients with relatively "pure" mitral stenosis.

5. This study suggests that the precordial electrocardiogram, when carefully correlated with the history, physical examination and roentgenologic findings, should be of definite value in detecting minor degrees of left ventricular hypertrophy in patients with dynamically significant mitral regurgitation in whom physical examination, cardiac fluoroscopy and pulmonary artery "wedge" pressures, alone or in combination, may fail.

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Causes of Death in Rheumatic Heart Disease*

Relationship to the Incidence of Mitral Stenosis Occurring Alone or with Other Valvular Lesions

LOUIS A. SOLOFF, M.D. and JACOB ZATUCHNI, M.D.

Philadelphia, Pennsylvania

LONG before as well as since the introduction of antibiotic therapy there has been a steady improvement in the prognosis of rheumatic fever and rheumatic heart disease.^{1,2} There continues, however, to be great variability and indeed unpredictability in the course of individuals afflicted with this disease. These differences in life histories are partly due, as in all inflammatory diseases, to differences in the intensity of the noxious agent and in the resistance of the host. In rheumatic individuals there are the additional factors of marked differences in the anatomic changes in the heart and in the complications or unavoidable accidents intimately connected with the nature of the cardiac disability.

No single therapeutic agent is effective in combating all the causes of disability or death. The time in the natural history of rheumatic heart disease when a particular disability and death due to it is most apt to occur may vary considerably. To determine the effectiveness of any therapeutic agent it is therefore necessary to know the natural history of the particular disability for which the agent is employed. To obtain a baseline for determining the effectiveness of new therapy we have analyzed the incidence, age, gross anatomic cardiac findings and cause of death in all individuals who died in our hospitals with a clinical diagnosis of rheumatic heart disease.

MATERIAL AND METHOD

An analysis was made of the records of all individuals with a clinical diagnosis of rheumatic heart disease who entered Temple University Hospital from 1945 to 1952 and Episcopal Hospital from 1945 to 1949, inclusive, and died. These intervals were chosen because

* From the Departments of Medicine, Temple University School of Medicine and Hospital, and Episcopal Hospital, Philadelphia, Pa.

1945 represents the beginning of the general availability of penicillin and many other antibiotics and because surgery for acquired valvular heart disease was started at Episcopal Hospital in 1949 and at Temple University Hospital in 1952. There were 141 such individuals. Of these there were autopsy records available in sixty-nine or 48.9 per cent. Several others were autopsied but were coroner's cases and the records were not available for study.

The clinical records were analyzed for the age at death, the clinical diagnosis with particular reference to rhythm, valvular lesions, the cause of death, complications directly related to the heart and the presence or absence of diseases other than those related specifically to rheumatic heart disease or its sequelae. The autopsy records were analyzed with particular reference to heart weight, valvular lesions, complications attributable to the cardiac lesions and the presence or absence of diseases not related to rheumatic heart disease.

RESULTS

The total number of admissions to both hospitals during this interval was 188,066; of these 5,807 (3.1 per cent) died. Of the 188,066 admissions 1,718 (0.91 per cent) had a clinical diagnosis of rheumatic heart disease and of this number 141 (8.2 per cent) died. The causes of death of these 141 individuals were divided into various groups according to the major clinical or autopsy findings, or both, considered by the various attending physicians to be directly responsible for the death of the individual:

1. *Acute Rheumatic Carditis.* Because of the present confusion of the clinical significance of the Aschoff body, no individual was included in

this group on the basis of necropsy findings alone. All had classical clinical manifestations of acute rheumatic infection with carditis. There were nineteen (13.5 per cent) such cases. Their ages at death varied from twelve to thirty-two years and averaged 20.9 years. Eight had a history of recurrent bouts of rheumatic fever. Ten had severe right heart failure. Fifteen had sinus rhythm and four had atrial fibrillation. One died after exploratory laparotomy for abdominal pain the cause of which was not determined. Another died of rheumatic pneumonitis shortly after delivery at full term. A clinical diagnosis of isolated mitral valve disease was made in twelve of the nineteen and of combined valvular disease in two. Of the remaining five, two were diagnosed as aortic regurgitation and three had no specific clinical diagnosis of valvular disease. These three had a diffuse precordial systolic murmur.

Autopsies were obtained in fourteen of the nineteen cases. Only three had isolated mitral valve disease, two of which were stenotic. One of these two had a slit-like mitral valve and the heart weighed 390 gm. The other, from a twenty-two year old girl who had rheumatic pneumonitis, had a mitral valve whose leaflets were described as glued together. Her heart weighed 285 gm. The remaining case of isolated mitral valve disease, a twelve year old boy, had a pancarditis with minimal involvement of the mitral valve and a heart that weighed 200 gm.

The other eleven patients autopsied had multi-valvular lesions and huge hearts. All had mitral valve involvement but only five showed stenosis. One of these five had calcification of the mitral valve in addition to widening of the aortic and tricuspid valves. Calcification of the mitral valve but without stenosis was present in one other patient who also had aortic valvular calcification.

Gross and microscopic examination revealed rheumatic pancarditis in all fourteen instances, with varying involvement of the layers of the cardiac wall. In addition, three had rheumatic pneumonitis and one had calcification of the left atrium.

Ten of the twelve with a clinical diagnosis of isolated mitral valve disease were examined at autopsy. The diagnosis was correct in only two. Four had associated aortic and tricuspid valve involvement, three aortic and one tricuspid.

Necropsy was performed in one of the two cases with a clinical diagnosis of aortic valvular

disease. Calcification was found in both the aortic and mitral valves but there was no definite stenosis.

The relatively largest hearts were found in the younger individuals, who also showed the most frequent occurrence of multiple valve involvement.

2. Bacterial Endocarditis. Sixteen of the 141 patients (11.3 per cent) died of bacterial endocarditis. All had positive blood cultures at some time during their illness. An enterococcus was isolated in three, a staphylococcus in two, *Salmonella choleraesuis* in one and *Streptococcus viridans* in the remainder. The group with bacterial endocarditis due to the last organism included three admitted in coma, three who died of heart failure and one of uremia after apparent cure of the infection. Serious complicating illnesses were present in ten. Three had cerebral hemorrhage, one brain abscess, one rheumatoid arthritis, one uremia, one hypertension, one hemiplegia, one aortic aneurysm and one was a chronic alcoholic.

The ages at death varied from fifteen to seventy-four years, with an average of 37.4 years. Fifteen had sinus rhythm and one atrial fibrillation. Ten were diagnosed as isolated rheumatic aortic disease, three as isolated mitral disease and three as combined valve disease.

Autopsies were obtained in thirteen of the sixteen cases. There were four with isolated mitral valve disease, only one of whom had stenosis. The size of the mitral orifice was $1\frac{1}{2}$ fingers and the valve was fibrotic. A focal infarct was present in the left ventricle that was 1.75 cm. thick. The heart weighed 350 gm. Clinically, there was a precordial systolic murmur. The other three cases had scarring of the mitral valve but no stenosis. Vegetations were present on the mitral leaflets in all four.

Two of the thirteen patients who were autopsied had isolated aortic valve disease. Seven had combined valve disease, the weight of the heart averaging 500 gm. Only one of this group had mitral stenosis. This occurred in the chronic alcoholic who also had aortic stenosis. Vegetations were present on both valves. He also had encephalomalacia.

Necropsy was performed in two of the three cases with a clinical diagnosis of isolated mitral valve disease. At autopsy one was found to have widening of the tricuspid and mitral valves with vegetations, the other had isolated aortic stenosis with calcification. Of the three pa-

tients who died of heart failure after sterilization of the blood stream, all had a clinical diagnosis of multivalvular lesions that was confirmed in the two with necropsies. The smallest mitral orifice was 8 by 10.5 cm.

3. Pulmonary Infarction and Heart Failure. This group consisted of nine individuals (6.4 per cent). Their ages at death varied from twenty-nine to fifty-three years and averaged 41.1 years. Heart failure was present for a variable period before death. One, fifty-three years old, had heart failure for nine years.

A clinical diagnosis of pulmonary infarction was made in only four of the nine. One of the others was diagnosed as pneumonia, one had bouts of hemoptysis regarded as due to congestion, one died suddenly on admission into the hospital, one had recurrent dyspnea for four years, and the remaining case had dyspnea and tightness in the chest for one year.

Six had sinus rhythm and three atrial fibrillation. Eight were diagnosed as isolated mitral valve disease. Of these eight, five had sinus rhythm and three atrial fibrillation. One case with sinus rhythm was diagnosed as combined mitral and aortic stenosis.

Necropsy was performed in seven of the nine cases. Only one had isolated mitral valve disease. The mitral orifice was thick, rigid and 4 cm. in circumference. The heart weighed 400 gm. Death was due to thrombosis of the right pulmonary artery with infarction of the right middle and lower lobes. This patient was twenty-nine years old and had been dyspneic for two years. The remaining six showed combined valve disease, always mitral and aortic and in two instances also tricuspid. In four of these six cases the mitral orifice was stenotic. The smallest admitted 1½ fingers and the largest was 8 cm. in circumference. One of these four was associated with aneurysmal dilatation of the left atrium and another with calcification of the mitral valve. Thrombi were found in the right atrium in one and in the left atrium in one; the former had sinus rhythm and the latter atrial fibrillation. All had large hearts that varied in weight from 360 to 600 gm. and averaged 501 gm.

Microscopically, the group had pathologic evidence (Aschoff bodies) of active rheumatic carditis.

4. Systemic Embolism and Pre-existing Heart Failure. Thirteen patients (9.2 per cent) died of this cause. Heart failure had been present for variable periods of time before the fatal embolic

episode. Four had a systemic embolic episode six months to nineteen years before a fatal recurrence. Eleven had cerebral emboli, one renal and one cerebral and pulmonary. Their ages at death varied from twenty-eight to fifty-nine years, averaging 47.7 years. Four had sinus rhythm and nine atrial fibrillation.

Six were diagnosed as isolated mitral valve disease, five in association with atrial fibrillation. Of these five, one had hypertension and one bronchiectasis. The other seven were diagnosed as combined valvular disease.

Autopsies were obtained in five of the thirteen cases. Isolated mitral valve disease was found in one, an individual fifty-three years old with hypertension and heart failure of five years' duration who was admitted in coma. The mitral orifice was 6 cm. in circumference and the left ventricle was hypertrophied. The heart weighed 500 gm. The clinical diagnosis in this instance was isolated mitral stenosis. Of the remaining four, three showed combined valve disease and one had aortic stenosis with calcification. The latter was diagnosed clinically as combined aortic and mitral disease. Of the other three, one who was clinically diagnosed as aortic stenosis, aortic regurgitation and mitral regurgitation had a dilated and calcified aortic valve and a moderately constricted mitral valve; one with a clinical diagnosis of combined aortic and mitral disease had a stenotic mitral orifice with calcification and also myocardial infarction due to an old coronary thrombosis; and the remaining case, similarly diagnosed clinically, had a moderately constricted mitral orifice with fibrosis of the tricuspid and aortic valves. All with combined valve changes or only aortic disease had large hearts that varied in weight from 490 to 600 gm. and averaged 498 gm.

5. Systemic Embolism without Heart Failure. Twelve individuals (8.5 per cent) belonged to this group. Their ages at death varied from twenty-seven to eighty-three years, averaging 46.5 years. Seven had cerebral emboli, one cerebral and common iliac, one cerebral and mesenteric, one had embolus to the aortic bifurcation and two popliteal. All had atrial fibrillation. There was a history of previous emboli in three instances, occurring one, two and three years previous to a fatal recurrence. Three had sudden collapse and died within days and one had sudden collapse and died in four weeks. Two had associated hypertension.

All were diagnosed as isolated mitral stenosis

and this was confirmed in the three who were autopsied. A thrombus was found in the left atrium in two of the three. All had slightly enlarged hearts that varied in weight from 390 to 400 gm. Each of the three had atrial fibrillation and cerebral embolism.

6. *Heart Failure.* This group included fifty-six individuals (39.7 per cent). Their ages at death varied from thirty to seventy-nine years and averaged 46.3 years. Sinus rhythm was present in twenty-nine and atrial fibrillation in twenty-seven.

A clinical diagnosis of isolated mitral valve disease was made in eighteen. Of these, eleven were thought to have mitral stenosis alone, five to have mitral stenosis and regurgitation, one mitral stenosis and interatrial septal defect, and the remaining one mitral regurgitation. The mean age of this group was 56.2 years. Thirteen of these eighteen had associated diseases, including uremia, coronary artery disease, hypertension, Kimmelstiehl-Wilson's syndrome, scleroderma, Lutembacher's syndrome, and chills and fever of unknown etiology.

Eight had a clinical diagnosis of isolated aortic valve disease. Of these, seven had aortic stenosis and one aortic regurgitation. Four had sinus rhythm and four atrial fibrillation. The mean age of this group was 57.8 years.

In one instance a clinical diagnosis of valve disease was not made until terminally.

Twenty-nine of the fifty-six cases had a clinical diagnosis of combined valve disease. The age at death averaged 45.8 years. Nine had associated diseases, including cerebral thrombosis, renal disease, jaundice of undetermined origin and bronchopneumonia. One died following laparotomy for possible intestinal obstruction.

Necropsy was performed in twenty cases. Only four showed isolated mitral valve disease, of which two were stenotic. One of the two, a thirty-three year old woman with scleroderma had a mitral valve fused into a fibrotic, trabeculated mass that had two 3-mm. openings. The other was a seventy-two year old man with hypertension and coronary artery disease. The heart of the former weighed 425 gm. and of the latter 620 grams. Both were diagnosed clinically as mitral stenosis. The remaining sixteen had combined valve disease of which thirteen had mitral stenosis and three of these were calcified. Another two had calcification but no definite stenosis. All had large hearts whose weight averaged 643 gm. Three of the thirteen with

mitral stenosis and associated valve lesions had other diseases. One had coronary artery disease, another hypertension and the remaining one had a laparotomy for possible intestinal obstruction but none was found.

Of the eighteen with a clinical diagnosis of isolated mitral valve disease, autopsy was obtained in seven. Of these, aortic and mitral valve disease was found in one; aortic, mitral and tricuspid disease in one; mitral and tricuspid in one; thickening of only the posterior leaflet of the mitral valve in one; thickening of the mitral valve but no stenosis in one, and mitral stenosis in the remaining two. Of these two, the mitral orifice was smallest in the patient with scleroderma; the other admitted 2 fingers. The latter had associated hypertension and coronary artery disease. In this group of seven cases the weight of the heart varied from 370 to 700 gm. and averaged 541 gm.

7. *Non-cardiac.* Sixteen individuals (11.3 per cent) died of causes unrelated to rheumatic heart disease or its sequelae. Their ages varied from twenty-six to seventy-three years old, averaging 46.3 years. Of the nine who were less than fifty years old at the time of death, two died of eclampsia, one of lower nephron nephrosis secondary to accidental injection of 15 cc. thiomerin, one of uremia and hypertension, one of multilobar pneumonia, two of bronchiectasis, one of cirrhosis and one of injuries incurred in an automobile accident. The others, all above the age of fifty at the time of death, died of such causes as gangrenous cholecystitis, uremia, and hypertension, myocardial infarction, pneumonia, cirrhosis and transfusion reaction.

Fourteen had sinus rhythm and two had atrial fibrillation. A clinical diagnosis of isolated mitral valve disease was made in eleven and multivalvular disease in four. One was diagnosed as aortic stenosis alone.

Necropsy was performed in seven and revealed isolated mitral valve disease in only two. Both of these had mitral stenosis without calcification. One was thirty-five years old and the other seventy years old. Both had enlarged hearts that weighed 325 gm. in the former and 580 gm. in the latter. The remaining five had combined valve disease. Of these, two had mitral stenosis and all showed cardiac enlargement.

Of the five with a clinical diagnosis of mitral stenosis who also had necropsies, combined valve disease was found in three.

The causes of death and the clinical and

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TABLE I
CAUSE OF DEATH OF INDIVIDUALS WITH RHEUMATIC HEART DISEASE WITH CLINICAL AND AUTOPSY FINDINGS OF VALVULAR INVOLVEMENT

Cause of Death	Total Group	Clinical		Autopsy		Mitral and/or Other Valve Involvement	Mitral Valve Involv. Alone	Mitral and/or Other Valve Involvement
		Mitral Valve Involvement Alone	Mitral and Other Involvement	Mitral	Aortic			
Rheumatic fever.....	19 13.5 12-32 20.9 15 4 12 63.2 21.5 8 4 1 2 10.5 26 2 0 0 3 2 14 73.4 3 2.0 1 292 1 11 11 5 2 9 1 1 7 11 574 0							
Bacterial endocarditis.....	16 11.3 15-74 37.4 15 1 3 18.8 34.3 3 0 2 3 18.8 37.3 3 0 2 0 10 13 81.3 4 1.0 3 335 4 9 7 1 0 7 3 1 4 9 500 5							
Pulmonary infarction and heart failure.....	9 6.4 29-53 41.1 6 3 8 88.9 38.8 5 3 2 1 11.1 53 1 0 0 0 0 7 77.8 1 1.0 1 400 0 6 6 4 1 6 1 3 2 6 501 1							
Systemic embolism and heart failure.....	13 9.2 28-59 47.7 4 9 6 46.2 49.2 1 5 2 7 53.8 45.1 3 4 0 0 0 5 38.5 1 1.0 1 500 1 4 3 1 4 3 2 1 4 498 1							
Systemic embolism.....	12 8.5 27-83 46.5 0 12 12 100.46.5 0 12 2 0							
Heart failure.....	56 39.7 30-79 50.9 29 27 18 32.1 56.2 12 6 13 29 51.8 45.8 12 17 9 1 8 20 35.7 4 2.0 4 454 3 16 13 5 14 7 6 4 16 643 4							
Non-cardiac.....	16 11.3 26-73 46.3 14 2 11 68.8 44.8 9 2 11 4 25.0 45.8 4 0 4 1 7 43.8 2 2.0 2 453 2 5 2 0 5 0 0 0 5 483 5							
Summary.....	141 12-83 41.5 83.5 80 49.7 41.6 38 32 33 46 32.6 42.2 25 21 15 4 21 69 48.9 18 12.0 15 404 11 51 48 28 9 45 15 13 18 51 533 16							

Episcopal Hospital 1945-1949 and Temple University Hospital 1945-1952

Total admissions.....	188,066	Individuals with rheumatic heart disease.....	1,718 (0.9%)
Total number of deaths.....	5,807	Number of deaths.....	141 (16)
Mortality rate.....	3.1%	Mortality rate.....	0.2% (7.3%)

autopsy findings of valvular involvement are shown in Table I.

Age Groups for the Various Causes of Death. This relationship is shown in Table II. Acute rheumatic fever and bacterial endocarditis caused all deaths in the second decade and the majority of

age of fifty. Ten were unrelated to rheumatic heart disease or its sequelae, nineteen died of acute rheumatic fever, fourteen of bacterial endocarditis, seven of pulmonary infarction and heart failure, fourteen of systemic embolism and twenty-eight of heart failure. An equal number

TABLE II
CAUSES OF DEATH IN THE VARIOUS AGE GROUPS

Age Groups:	12-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	Total
Acute rheumatic fever.....	11	5	3	19
Bacterial endocarditis.....	2	3	5	4	1	..	1	..	16
Pulmonary infarction and heart failure.....	..	2	3	2	2	9
Systemic embolism and heart failure.....	..	1	3	2	6	1	13
Systemic embolism.....	..	2	3	3	1	2	..	1	12
Heart failure.....	..	2	12	14	15	9	4	..	56
Non-cardiac.....	..	4	2	4	2	3	1	..	16
Total.....	13	19	31	29	27	15	6	1	141

TABLE III
RELATION OF NECROPSY FINDING OF ISOLATED MITRAL STENOSIS TO AGE AND CAUSE OF DEATH

Age Groups:	12-20	21-30	31-40	41-50	51-60	61-70	71-80	Total
Acute rheumatic fever.....	..	1	1	2
Bacterial endocarditis.....	1	1
Pulmonary infarction and heart failure.....	..	1	1
Systemic embolism and heart failure.....	1	1
Systemic embolism.....	..	1	2	3
Heart failure.....	1	1	2
Non-cardiac.....	1	1	..	2
Total.....	..	3	5	..	2	1	1	12

those in the third. In this series, no individual died after the age of thirty-two with classical clinical manifestations of acute rheumatic fever. We have, however, previously commented upon the microscopic picture of the myocardium in those who died of pulmonary infarction and heart failure.

Ninety-two of the 141 patients died before the

of individuals died of heart failure after the age of fifty. The oldest individual in this series was eighty-three years and he died following a mid-thigh amputation for popliteal embolism. He had mitral stenosis and regurgitation with atrial fibrillation.

Ages for the Various Groups of Individuals with Necropsies Showing Isolated Mitral Stenosis. This

group included twelve individuals, eight of whom died before the age of fifty. Of these, two died of acute rheumatic fever, one of pulmonary infarction and heart failure, three of cerebral embolism, one of heart failure and one of lower nephron nephrosis. The only one who died of heart failure before the age of fifty was the thirty-three year old woman with scleroderma.

failure, two of systemic embolism and heart failure and the other eight of heart failure. These relationships are shown in Table IV.

COMMENTS

It is interesting to note that of the total number of admissions, 0.9 per cent (1,718 of 188,066) entered our hospitals with a clinical diagnosis of

TABLE IV
RELATION TO AGE AND CAUSE OF DEATH OF THE NECROPSY FINDING OF MITRAL STENOSIS IN PRESENCE OF OTHER VALVULAR LESIONS

Age Groups:	12-20	21-30	31-40	41-50	51-60	61-70	71-80	Total
Acute rheumatic fever.....	1	3	1	5
Bacterial endocarditis.....	1	1
Pulmonary infarction and heart failure.....	2	..	2	4
Systemic embolism and heart failure.....	..	1	2	3
Systemic embolism.....
Heart failure.....	..	1	3	1	6	1	1	13
Non-cardiac.....	..	1	..	1	2
Total.....	1	6	7	2	10	1	1	28

Four died after the age of fifty years, one of bacterial endocarditis, one of systemic embolism and heart failure, one of heart failure and the remaining one of alcoholic cirrhosis. The patient who died of heart failure was seventy-two years old and had hypertension and coronary artery disease with an enlarged heart that weighed 620 gm. The mitral orifice admitted 2 fingers. These age relationships are shown in Table III.

Age Groups for the Various Causes of Death in Individuals with Necropsies That Showed Mitral Stenosis and Other Valve Involvement. This group included twenty-eight individuals. There were sixteen below the age of fifty years, five of whom died of acute rheumatic fever, one of bacterial endocarditis, two of pulmonary infarction and heart failure, one of systemic embolism and heart failure, five of heart failure, one of hypertension and uremia and the remaining one of suppurative pulmonary disease. Of the five who died of heart failure, all had severe aortic valve disease. Calcification of the aortic valve was present in two and of the mitral valve in one.

There were twelve patients who died after the age of fifty, two of pulmonary infarction and heart

rheumatic heart disease, frequently with associated unrelated illnesses. Less than 1 per cent of these individuals died and 39.1 per cent of those dead of rheumatic heart disease or its sequelae had associated significant but unrelated illnesses such as hypertension, bronchiectasis, uremia, myocardial infarction, diabetes mellitus, rheumatoid arthritis, jaundice, scleroderma or infection.

Some may be surprised that we had only 141 deaths of individuals with a clinical diagnosis of rheumatic heart disease. Individuals diagnosed at necropsy as rheumatic heart disease not suspected clinically are not included in this study. Different hospitals differ in population, ratio of medical to surgical cases and other factors, but it is perhaps interesting to note our number in comparison with that reported by others. Laws and Levine³ were able to find only 148 cases of rheumatic heart disease in which a postmortem examination was performed at the Peter Bent Brigham Hospital from 1913 to 1932. Juca and White⁴ reported on 100 unselected fatal cases over the age of twenty years who were examined at the Massachusetts General Hospital

between the years 1927 and 1943 inclusive. Graham et al.⁵ found 100 cases with rheumatic heart disease and mitral valve involvement in the postmortem files of the Mallory Institute of Pathology of the Boston City Hospital between January, 1945, and June, 1949. Finally, Edstrom and Gedda⁶ found 353 cases at a large hospital in Sweden for the years 1934 to 1951. It appears, therefore, that our experience is not very different from that of other general hospitals. Yet, the crude death rate for rheumatic heart disease in our hospital population is 74.4 per 100,000 as compared to 16.0 per 100,000 for all deaths due to rheumatic fever, chorea and chronic rheumatic heart disease in the entire population of the United States in 1950.¹

It is important to remember that our analysis is based on deaths only, a form of selection, as pointed out by Wilson⁷ that is "the severest type of sampling bias, since it is only the persons who die earliest, i.e., the poorest risks, who are used to determine the prognosis figures." We may add that deaths in hospitals indeed represent a more selected sampling than deaths as a whole because older patients and those who have chronic illness, or have been previously repeatedly hospitalized and have failure that appears intractable, more commonly die at home. This is illustrated by the fact that our average age at death for the clinical group was 41.5 years and for the necropsy group 48.9 years whereas the average age of death for all rheumatic individuals in the United States in 1950 was 55.5 years.⁸ Any therapy, therefore, which is regarded as effective so far as life is concerned must be shown to surpass by a wide margin the mean ages of these various groups.

In our series, acute rheumatic carditis was the second most common cause of death. Its incidence is undoubtedly underestimated because of two reasons. First, no individual was included in this group on the basis of necropsy findings alone, although some who died of heart failure had large hearts, Aschoff bodies and other changes that ordinarily would be considered as active rheumatic carditis.⁹ Secondly, the incidence was further decreased by excluding the nine individuals who died of pulmonary infarction and heart failure. Several of these individuals had clinical and necropsy findings that simulated those found in rheumatic pneumonitis and all had myocardial Aschoff bodies.

Bacterial endocarditis accounted for 11.3 per cent of the deaths. This incidence may be com-

pared to the 29 per cent reported by Laws and Levine³ 18 per cent by Juca and White,⁴ 11 per cent by Edstrom and Gedda⁶ and also by Graham et al.⁵ Bacterial endocarditis continues to be a serious problem because of the increasing incidence of offending organisms other than the *Streptococcus viridans*, the abrupt onset with coma, the serious complications such as brain abscess or hemorrhage and the occurrence of congestive failure or uremia after successful sterilization of the blood stream.

Individuals dying of systemic emboli were divided into two groups depending upon the presence or absence of pre-existing heart failure. It is possible that in those with heart failure, emboli might arise, as often happens in failure of other causes, from peripheral veins or from local stasis, whereas in those without failure, emboli might arise from the left auricle or be due to atrial fibrillation or both. Indeed, differences were found in that all those with emboli without failure had isolated mitral stenosis whereas those with failure had predominant combined valve disease.

In the group with heart failure alone, it is noteworthy that a diagnosis of isolated mitral stenosis as a cause of fatal congestive failure is most often wrong. There were only two such instances, one in an individual seventy years old and the other in a woman thirty-three years old, with advanced scleroderma.

Indeed, a diagnosis of isolated mitral stenosis is often wrong for any group except the one with fatal systemic embolization without heart failure. Forty-nine and seven tenths per cent had a clinical diagnosis of isolated mitral valve disease that was corroborated in only 26.1 per cent at necropsy. This figure is remarkably similar to that of 25 per cent found by Laws and Levine.³ The error in the clinical diagnosis of isolated mitral valve disease is also similar to that of Edstrom and Gedda.⁶ We wish to stress, however, that the error of a clinical diagnosis of isolated mitral valve disease is greatest for those dying of congestive failure. Indeed, its rarity is shown by the fact that Edstrom and Gedda could find only three instances a year of individuals with isolated mitral stenosis regardless of the cause of death. Laws and Levine found only nine instances in nineteen years, less than one every two years which, considering the number of autopsies, is practically identical with our incidence of isolated mitral valve disease. Levine made no distinction between mitral valve disease

and mitral stenosis such as was made later by Dana.¹⁰ It is also noteworthy that the average age of death found by Laws and Levine was 48.1 years. However, the average age of death for their entire group with congestive failure was 40.5 years whereas ours was 46.3 years. Perhaps the improved prognosis may provide more time for development of other valvular lesions and so continually decrease the incidence of isolated mitral stenosis as a cause of fatal congestive failure. It is perhaps more than coincidental that multivalvular lesions are commonly present both in individuals dying of active rheumatic carditis and those dying of congestive failure.

Finally, it is noteworthy that calcification of the mitral valve was not found in this series as an isolated valve lesion.

CONCLUSIONS

1. Of 188,066 admissions to Temple University Hospital and Episcopal Hospital during the years 1945 to 1952 and 1945 to 1949, respectively, 141 with a clinical diagnosis of rheumatic heart disease died.

2. The causes of death, in decreasing order of frequency, were heart failure (39.7 per cent), acute rheumatic carditis (13.5 per cent), bacterial endocarditis (11.3 per cent), systemic embolism and pre-existing heart failure (9.2 per cent), systemic embolism without heart failure (8.5 per cent) and pulmonary infarction and heart failure (6.4 per cent). In the remaining 11.3 per cent death was not related to rheumatic heart disease or its sequelae.

3. Diseases in addition to rheumatic heart disease were present in at least 39.1 per cent.

4. Acute rheumatic fever or bacterial endocarditis caused all of the deaths before the age of twenty years and the majority of cardiac deaths in the third decade.

5. Of those who died of heart failure, as many died before as after the age of fifty.

6. A clinical diagnosis of isolated mitral valve disease was made in 49.7 per cent but this diagnosis was confirmed at necropsy in but 26.1 per cent. Isolated mitral stenosis was found in only twelve individuals (17.4 per cent).

Mitral stenosis in combination with other valvular lesions was found in twenty-eight (40.6 per cent).

7. All those with systemic emboli without heart failure had isolated mitral stenosis whereas those with failure had predominant combined valve disease.

8. Only four individuals with isolated mitral valve disease had fatal heart failure, two of whom had stenosis. One was thirty-two years old and had scleroderma, the other was seventy-two years old and had hypertension and coronary artery disease.

9. Calcification of the mitral valve was found at necropsy in 13.0 per cent but never occurred in the presence of isolated mitral valve disease.

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Observations on the "Juvenile Pattern" of Adult Negro Males*

RICHARD H. WASSERBURGER, M.D.

Madison, Wisconsin

As part of a study of the effect of pulmonary resection on the cardiovascular system all patients at the Veterans Administration Hospital, Madison, Wisconsin, have undergone serial electrocardiography. On review of these records our attention was directed to the common occurrence of an unusual pattern in the adult Negro male. In brief, this consists of frank inversion of the T waves in the right and mid-precordial leads with lesser change reflected in the standard and augmented limb leads. Because of the close similarity in T wave configuration of this particular pattern to that seen in infants and young children, it has been referred to as the "juvenile pattern." It should be emphasized that the similarity exists only in the T waves and is not reflected in QRS complex configuration. The T wave inversion pattern as seen in infants and young children is rarely present after the age of ten years and, as a rule, is on the wane after the age of four years.¹⁻⁴ In the recent literature the occurrence of this pattern has been questioned. The present material is presented in an attempt to establish the "juvenile pattern" as a normal variant in the adult Negro male. This is supported by a study of the effects of potassium salts, pro-banthine^{®†} and hyperventilation on the electrocardiograms of fourteen adult Negro males who exhibited variances of the "juvenile pattern" on routine admission electrocardiograms.

METHODS AND MATERIALS

All patients in this study were hospitalized for definitive therapy for tuberculosis and, with one exception, the tuberculous process was pulmonary in nature. Once the electrocardiographic

† Pro-banthine, bromide, brand of propantheline bromide, G. D. Searle & Company, Skokie, Ill.

pattern was clearly established the patient was subjected to critical cardiac investigation, including detailed history and physical examination. Serial teleoroentgenograms and orthodiascopy revealed normal cardiac silhouettes in all instances. Particular care was exercised to rule out clinical evidence of pericarditis. All patients were normotensive. The age range was from twenty-six to forty-eight years with a mean of thirty-three years. Ten of the fourteen patients were unusually tall and all but one were of an asthenic habitus. All of the subjects readily professed to being tense individuals, with many admitting a profound fear of the electrocardiograph which could not be resolved by repeated reassurance. The electrocardiograms were performed in the recumbent position with a direct writing electrocardiograph, utilizing the standard and augmented limb leads and six or more unipolar chest leads.

Twelve of the fourteen patients studied received an oral preparation of potassium citrate and potassium bicarbonate in a dosage of 5 gm. each. The serum potassium levels were performed with a model DU flame spectrophotometer. Control electrocardiograms and serum potassium levels were obtained immediately prior to the administration of potassium. Following drug intake the electrocardiograms and serum potassium levels were taken at thirty, sixty and ninety minutes. After completion of the initial study progress electrocardiograms were obtained at intervals during the remainder of the patients' hospitalization.

Ten patients, in whom suitable control tracings could be obtained, were subsequently given 20 to 30 mg. of pro-banthine intravenously in an effort to study the effect of a suitable vagolytic blocking agent on the "juvenile pattern." Prior to the injection of the pro-banthine hyperventila-

* From the Department of Medicine, Cardiology Section, Veterans Administration Hospital, and Department of Medicine, University of Wisconsin School of Medicine, Madison, Wis.

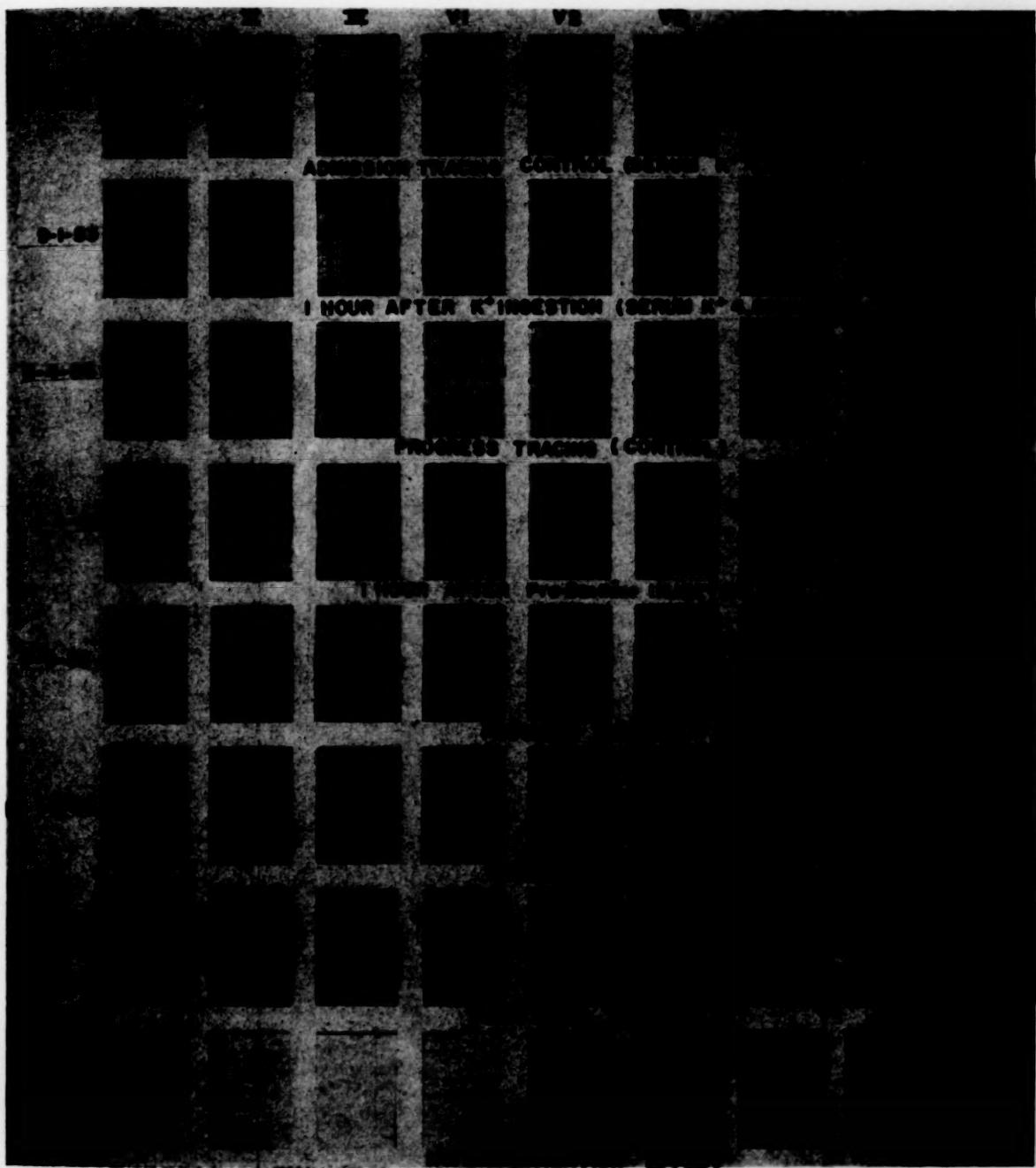


FIG. 1. Case 8.

tion was carried out for 10 to 20 seconds. The drug was given in 10 cc. of fractionally distilled water over a two- to four-minute injection period. Continuous electrocardiographic monitoring was carried out until the initial tachycardia had stabilized near 145–150/min. Progress electrocardiograms were taken at 30, 60, 120 and 180 minutes from time of injection. Serum potassium specimens were drawn prior to the drug administration and one and a half hours

thereafter. Hyperventilation was repeated after maximum pro-banthine effect was noted, which usually occurred within one to one and a half hours after injection.

REPRESENTATIVE CASE REPORTS

CASE 8. C. R., a thirty-four year old man, was admitted to this hospital with the diagnosis of lymphohematogenous tuberculosis as verified by cervical lymph node biopsy. His admission

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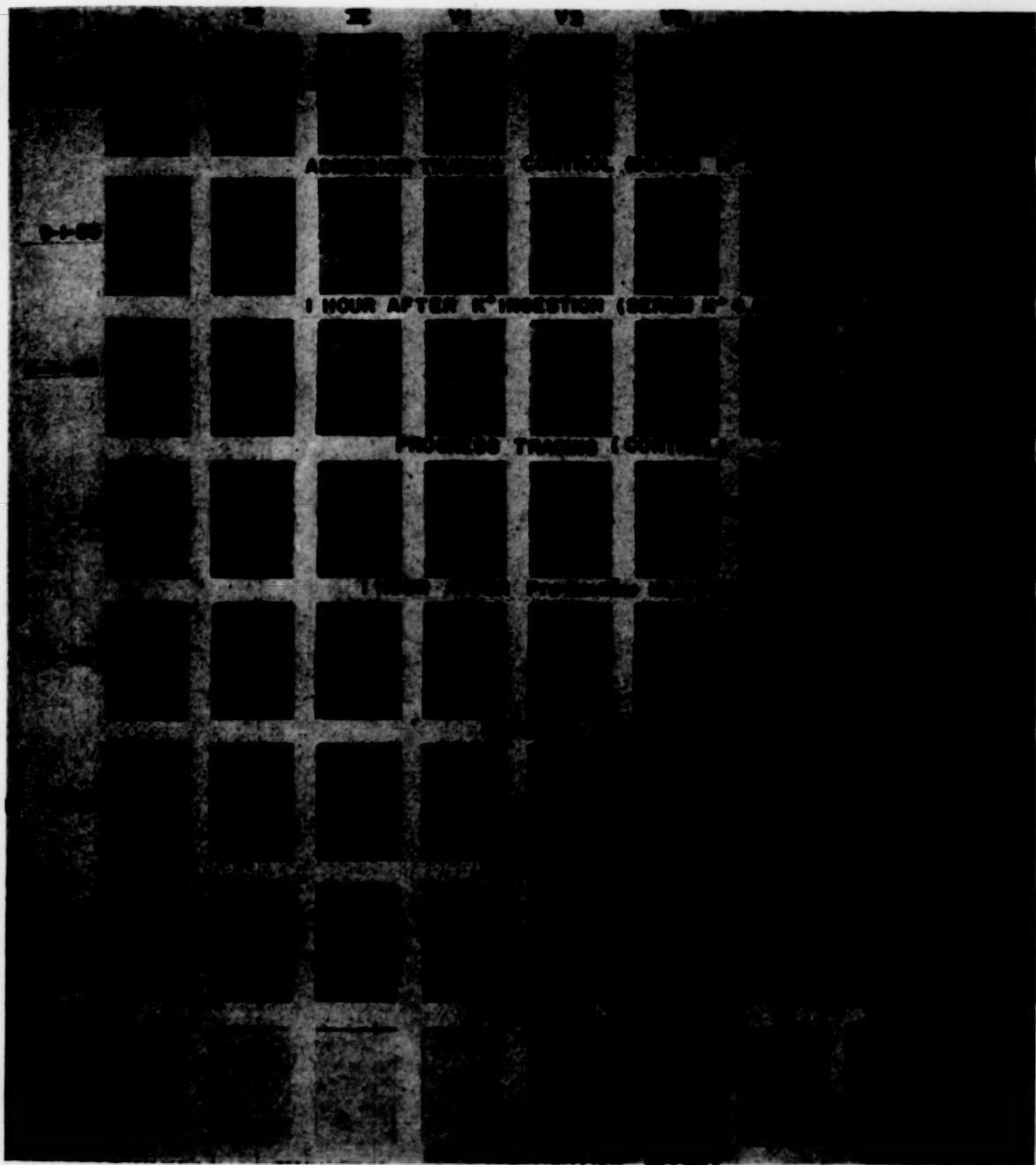


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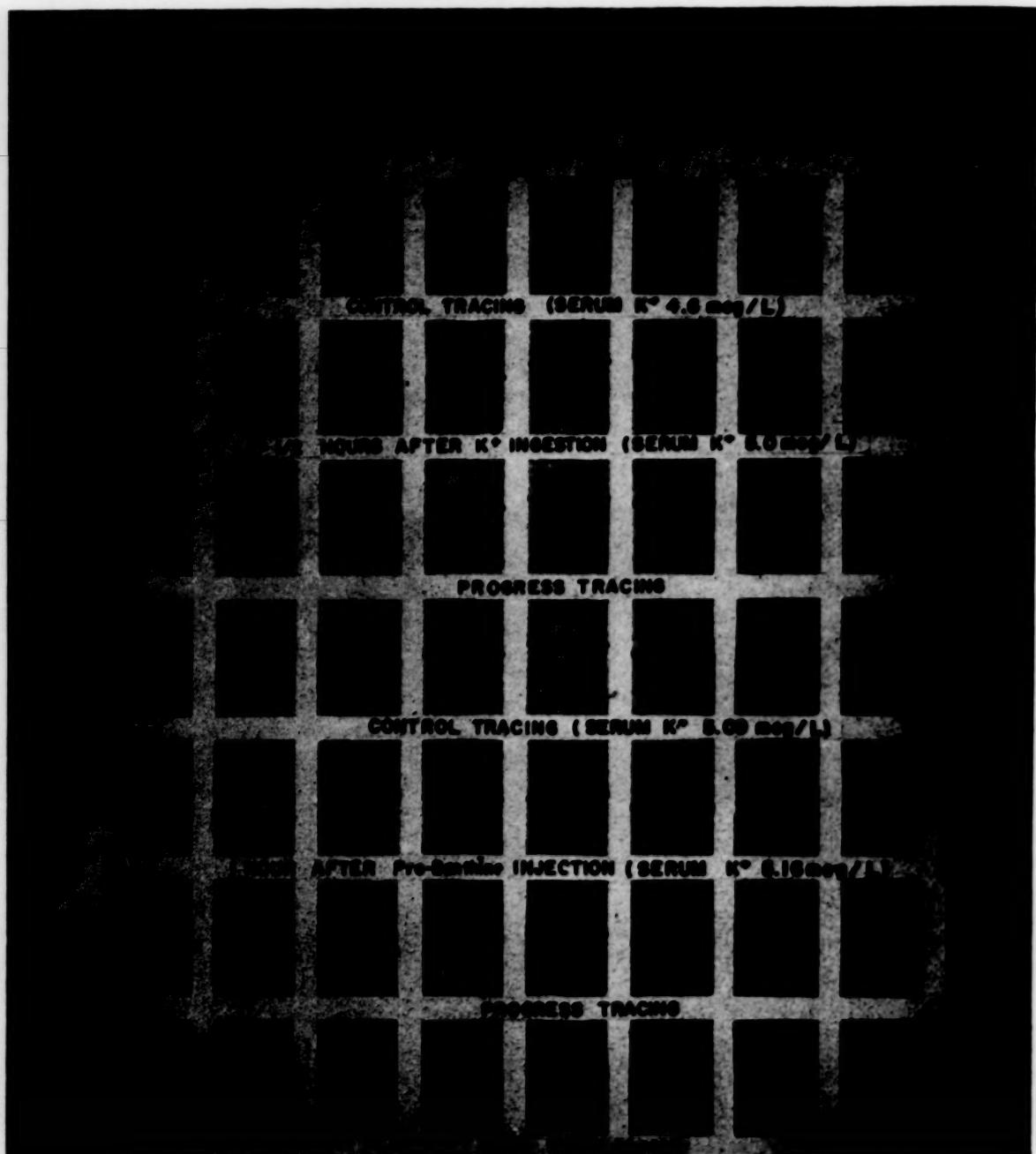


FIG. 2. Case 9.

chest film disclosed minimal infiltrative disease involving the posterior basilar segment of the left lower lobe. His admission tracing on September 1, 1953, showed frank T wave inversion across the entire precordium with flattening of T-1 and T-2. The tracing taken on September 1st following the oral ingestion of potassium was readily within normal limits. A progress tracing on September 4th disclosed only minimal terminal inversion of T-V₂ through T-V₄. The tracing

taken the same day following pro-banthine injection was clearly within normal limits, as was a progress tracing on September 9th. The tracing of October 14th once again disclosed terminal T wave notching in the right and mid-precordial leads. The T wave inversion in leads V₂, V₃ and V₄ was readily accentuated following hyperventilation as noted on the tracings of December 22nd, and was comparable to the admission tracing of September 1st. The patient

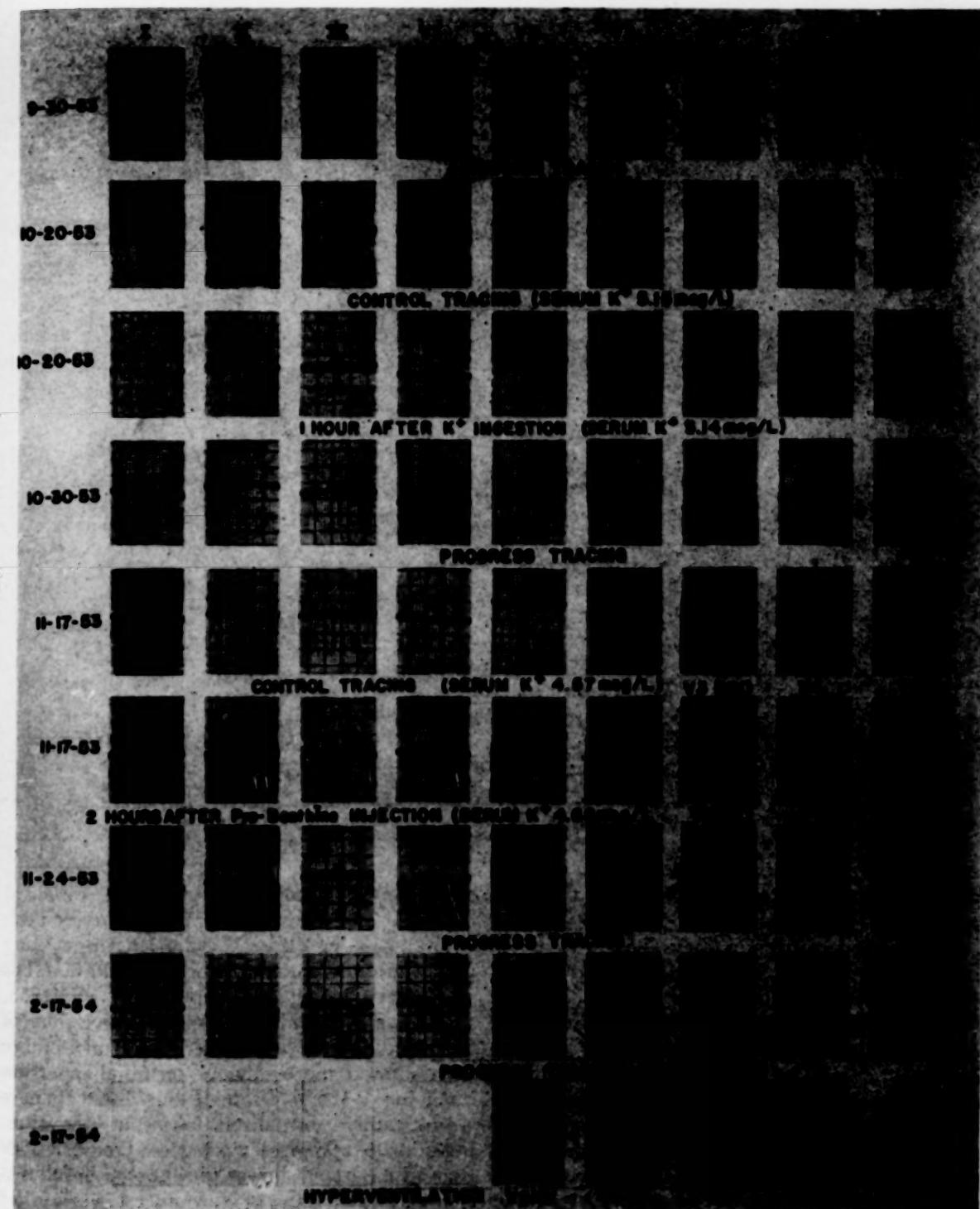


FIG. 3. Case 10.

was an extremely suspicious and immature individual. (Fig. 1.)

CASE 9. C. A. was a thirty-three year old man, whose admission chest film disclosed minimal nodular disease in the left apex. The patient

was a profound psychoneurotic and on several occasions there was a question of a frank psychotic episode. His admission tracing of September 8, 1953, showed marked T wave inversion in all of the standard and unipolar

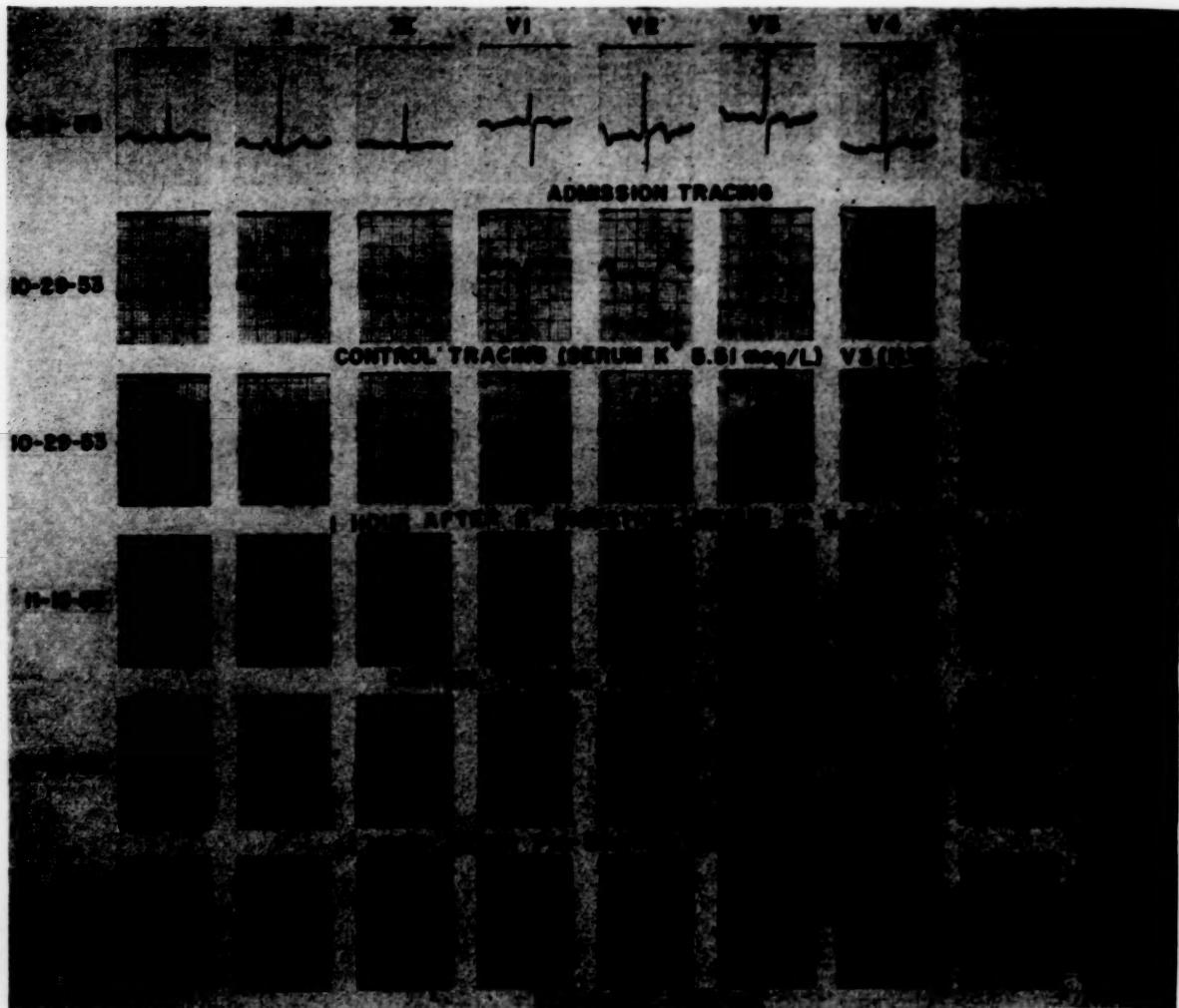


FIG. 4. Case 11.

leads extending over to and including V_6 . The tracing taken on September 10th, one and a half hours following potassium ingestion, disclosed the T waves to be generally upright. A progress tracing on October 16th showed a lesser degree of T wave inversion. The control tracing on November 12th again showed marked T wave inversion throughout precordial leads, with the tracing taken one hour after pro-banthine injection disclosing upright T waves throughout. The progress tracing on November 20th was entirely similar to that noted at time of hospital admission. The tracing of January 6, 1954, taken several weeks after left-sided thoracotomy, was clearly within normal limits. At the time of left-sided thoracotomy the pericardium was smooth and glistening, with no evidence of pericarditis. A small, isolated apical tuberculous focus was resected. (Fig. 2.)

CASE 10. I. S. was a twenty-six year old

man, whose admission chest film disclosed bilateral far advanced pulmonary tuberculosis with the major disease in the left upper lung field where there was evidence of cavitation. His admission tracing on September 30, 1953, showed only minimal terminal inversion of $T-V_2$ and $T-V_3$. The control tracing on October 20th showed more obvious terminal inversion involving $T-V_2$, $T-V_3$ and $T-V_4$. These changes were readily normalized following potassium ingestion. A progress tracing on October 30th showed a pattern similar to that seen on admission with the tracing of November 17th showing marked accentuation of the T wave inversion pattern in lead V_2 , $H.V.$ following hyperventilation. This effect was readily blocked following pro-banthine injection and the T waves in general were of normal amplitude and configuration. The tracing of November 24th showed reversion to the original pattern. The

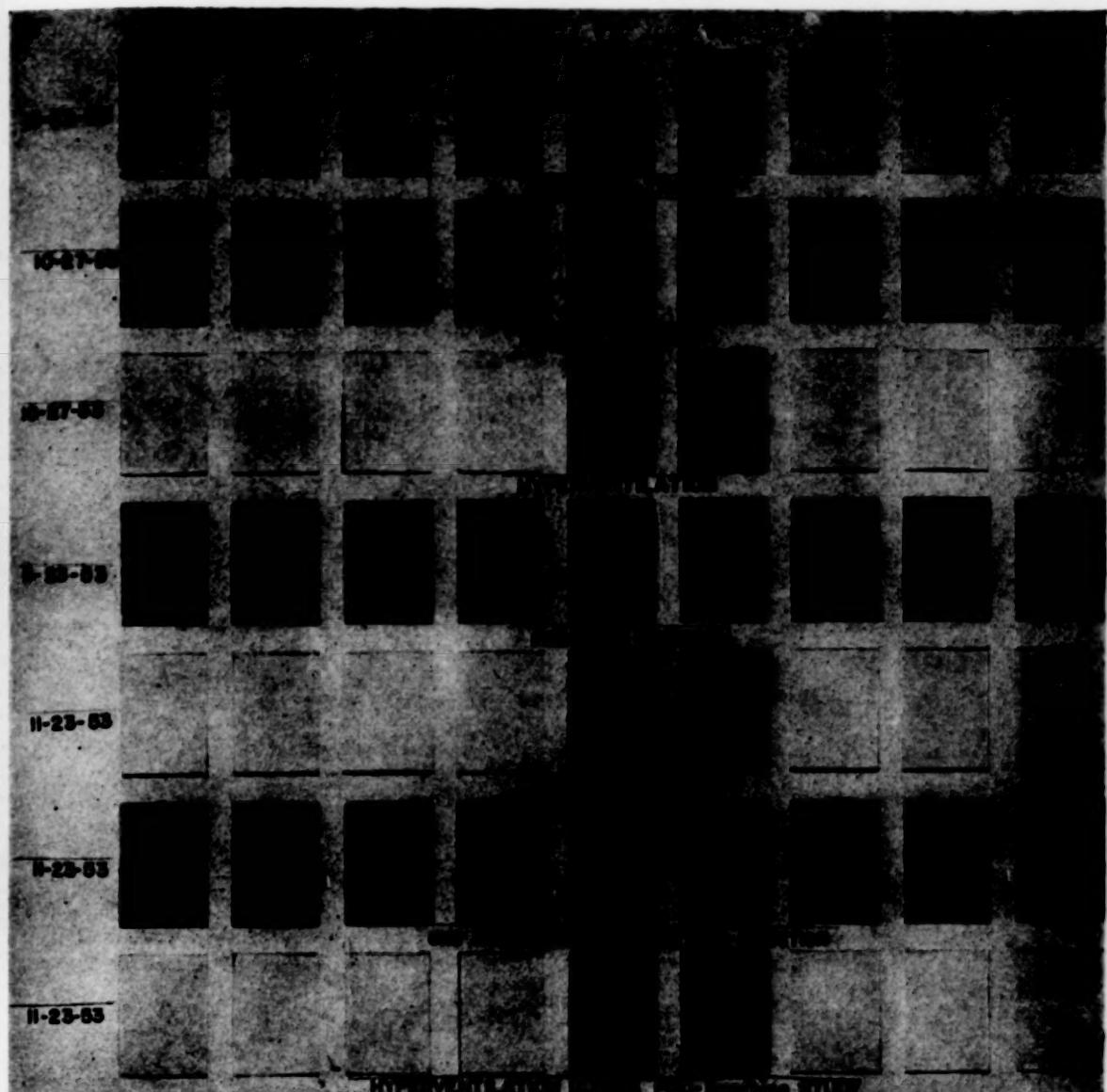


FIG. 5. Case 12.

patient's most recent tracing of February 17th was clearly within normal limits with prompt inversion of T-V₂, T-V₃, T-V₄ and T-V₅ with hyperventilation. (Fig. 3.)

CASE 11. J. B. was a twenty-five year old man, whose admission chest film disclosed moderately advanced right upper lobe tuberculosis with cavitation. Partial bronchial stenosis was noted at time of bronchoscopy. His admission tracing on October 22, 1953, disclosed inversion of T-V₂ and T-V₃. The progress tracing on October 29th was within normal limits; however, T-V₃ became markedly inverted during hyperventilation. The tracing taken following potassium ingestion was clearly within

normal limits. A progress tracing on November 16th disclosed slight terminal T wave inversion in leads V₂ and V₃ which was readily abolished following pro-banthine injection. A progress tracing on November 24th was once again within normal limits. Initially, the patient was openly hostile; however, during the latter period of study he became much more friendly and cooperative. (Fig. 4.)

CASE 12. This patient, R. W., was twenty-nine years of age. Admission chest film disclosed moderately advanced pulmonary tuberculosis involving the right upper lobe which was partially atelectatic. The patient was a profound psychoneurotic with a psychopathic personality.

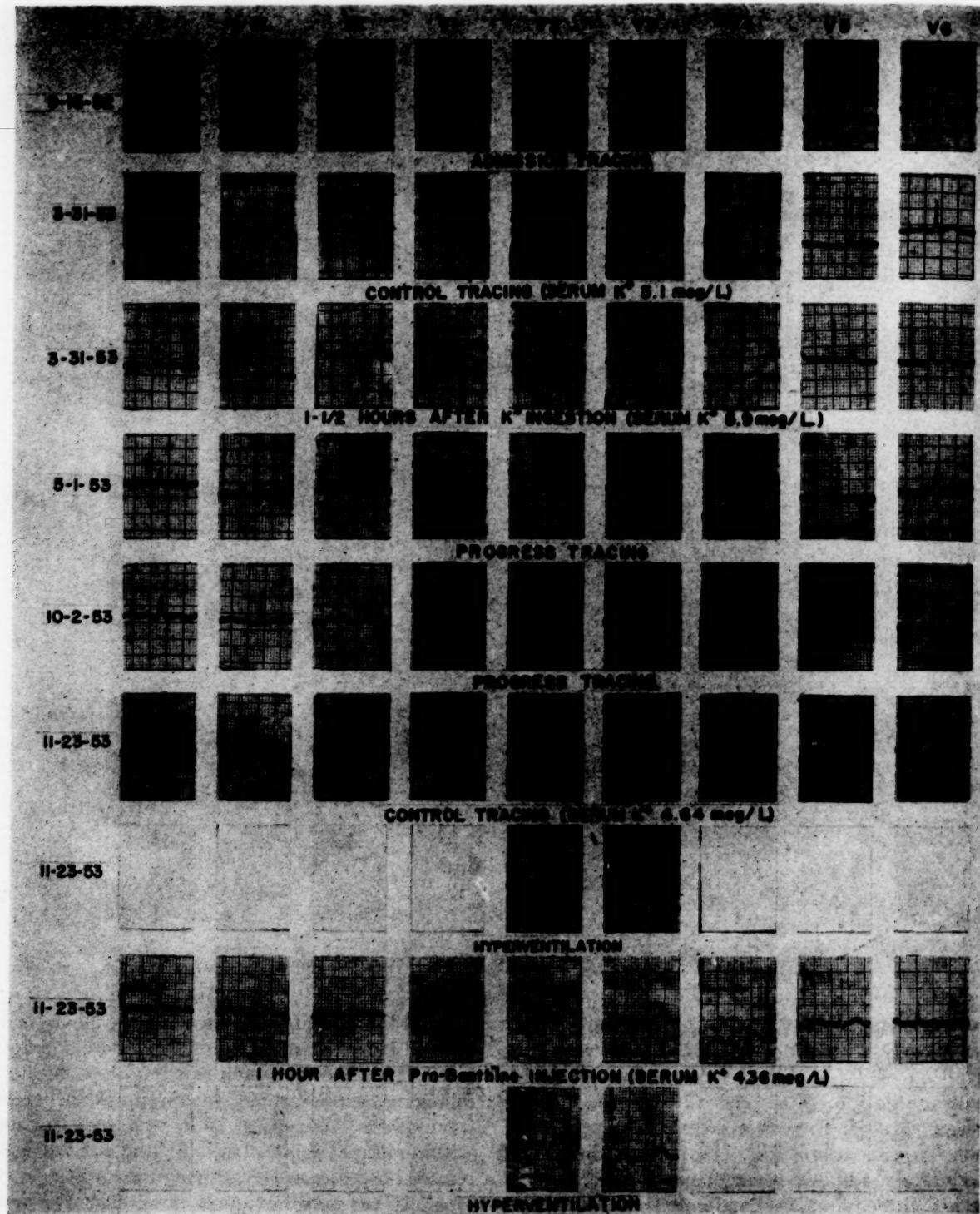


FIG. 6. Case 14.

Admission tracing of October 22, 1953, disclosed frank inversion of T-V₂ and T-V₃. A progress tracing on October 27th was within normal limits, with marked inversion of T-V₂ and T-V₃ during hyperventilation (V₂H.V.-

V₃H.V.). A control tracing on November 23rd showed similar findings as regards T wave inversion with hyperventilation and this effect was readily blocked following pro-banthine injection. Potassium salts were not administered

to this patient because a suitable control tracing could not be obtained. The patient was given a disciplinary discharge from the hospital on February 19, 1954, because of repeated infraction of hospital policy. (Fig. 5.)

CASE 14. A. H., age thirty-six, had known pulmonary disease since February, 1952. Admission x-ray showed far advanced pulmonary tuberculosis, chiefly involving the right upper lobe where there was evidence of cavitation. Pleural thickening was noted at both lung bases, more marked on the right. Admission electrocardiogram on September 15, 1952, disclosed rather marked T wave inversion in Leads V₃ and V₄ with flattening of T-₁, T-₂ and T-V₅. The control tracing of March 31, 1953, showed a basically similar pattern. The tracing taken one and a half hours following potassium ingestion on the same day was within normal limits, disclosing only slight terminal notching of T-V₃ and T-V₄. A progress tracing on May 1st showed a pattern similar to that at time of hospital admission. A right upper lobectomy with decortication was carried out on September 10th. The routine tracing on October 2nd was within normal limits, save for slight notching of T-V₃ and T-V₄. A control tracing on November 23rd was within normal limits although hyperventilation at this time resulted in prompt inversion of T-V₂H.V. and T-V₃H.V. This effect was readily blocked following pro-banthine injection. (Fig. 6.)

RESULTS

Of the 131 Negro patients admitted to this hospital between May, 1952, and October, 1953, there were fourteen who exhibited T wave changes consistent with the "juvenile pattern." This represents an incidence of 10.8 per cent of the total Negro population. Of a total of 550 Caucasian males studied during this same period, only two showed a similar pattern.

Pertinent historical data and electrocardiographic tracings observed in this study are seen in Figures 1 through 6.

The twelve patients given oral potassium salts showed normalization of the T wave pattern within one and a half hours following ingestion. (By normalization is meant that the T waves assumed an upright configuration of normal amplitude.) The serum potassium levels were normal in each instance during the control period. The serum levels rose slightly following administration of potassium but in

only two cases was an abnormally high potassium level observed. There were no instances of conduction defect or arrhythmia following the administration of potassium. The mixture of potassium citrate and bicarbonate proved palatable and none of the patients complained of gastrointestinal symptoms.

Ten of the patients received intravenous pro-banthine. The T waves became upright in each instance and reached maximum amplitude between sixty and ninety minutes after time of the injection. A moderate degree of tachycardia was the rule (125 to 145 per minute), one patient showing an immediate response of 180 beats per minute. No untoward reactions were detected although xerostomia and mild mydriasis persisted for four to six hours. By the end of a three-hour period the original pattern began to reappear. The serum potassium levels were not influenced appreciably by the administration of pro-banthine.

Hyperventilation, which in the control tracings would promptly accentuate the "juvenile pattern" and on occasion make it manifest when it had transiently disappeared, did not affect T wave change following the administration of pro-banthine. U waves, which were common in the control studies, were noted infrequently during the potassium and pro-banthine study periods.

COMMENTS

The demonstration by Goldberger⁶ that orally administered potassium quite consistently abolished the T inversion in the precordial leads of children prompted its use in the present study. As in children, a temporary normalization of the "juvenile pattern" followed the administration of potassium, an effect which was accomplished without a marked increase in serum potassium levels. It is not implied, however, that this demonstration in itself negates the significance of the T wave inversion, for orally administered potassium will frequently normalize the inverted T waves associated with left ventricular hypertrophy, infarction patterns, myxedema and beriberi.⁶⁻⁸ Reynolds⁹ and Merrill¹⁰ reported that the critical serum potassium level for T wave inversion is 3 mEq./L. Reynolds believes that serum calcium, sodium and chloride ion concentrations only rarely influence the T wave pattern and he could find no correlation between the CO₂ combining power and the T wave amplitude. Bellet¹¹ has documented cases

of hypokalemia with electrocardiographic patterns simulating acute pericarditis and subepicardial myocarditis. Similar precordial T wave inversion patterns have been observed following vagal stimulation via carotid body massage,¹² and in cases of funnel chest deformity.¹³ Pertinent experimental data have shown that cardiac muscle ischemia associated with infarction and congestive heart failure results in a fairly marked increase in potassium content of coronary sinus blood as well as a decrease in myocardial potassium content.¹⁴⁻¹⁶

The occurrence of the "juvenile pattern" in apparently normal adult Negroes was first reported by Littman.¹⁷ Utilizing the bipolar leads, he found that approximately 5 per cent of adult Negroes demonstrated T wave inversions from CF₁ to CF₄. Only a single patient exhibited similar findings in a control study of 200 Caucasian adults. Littman's findings have been confirmed by Goldberger,¹⁸ Myers¹⁹ and Sokolow,²⁰ but denied by Kossman,²¹ Ashman²² and more recently by Keller and Johnson.²³ Suarez and Suarez, Jr.²⁴ have found similar T wave inversion patterns in Puerto Ricans, utilizing the Wilson unipolar leads. They interpreted these changes as being evidence of intrinsic myocardial disease if found in any patient above the age of nineteen years.

In view of the controversy existing over this pattern, the relatively high incidence (10.8 per cent) of the "juvenile pattern" in the present series is surprising. It may well be that the prospect of long term hospitalization, with its inherent emotional problems, has weighted the statistical findings as compared to an outpatient study. The exact significance of the "juvenile pattern" in adult Negroes is unknown, but from this study it is apparent that orally administered potassium salts, as well as intravenous pro-banthine, promptly resulted in normalization of this pattern. These changes observed under drug control were of a temporary nature, with peak effect noted approximately one hour from the time of administration of either agent. The accentuation of the inverted precordial T wave patterns by hyperventilation was consistently blocked by pro-banthine injection.

On the basis of the present study the exact mechanism for these changes cannot be definitely stated, particularly from the cellular biochemical aspect, but it is suggested that hypervagotonia may be intimately related to the "juvenile pattern." All of the patients studied

were anxious, tense individuals who were particularly fearful of the electrocardiograph procedure. Vasomotor instability was a consistent finding. Three of the patients were profound psychoneurotics with rather fixed hypochondriacal tendencies.

It is important that the changes identified as the "juvenile pattern" be considered a functional cardiac variation in Negro patients who have a high index of emotional instability, rather than denoting frank intrinsic myocardial disease. This is clearly evident when it is recalled that of the three patients who had previous electrocardiograms before entering this hospital, two had the electrocardiographic diagnosis of "subacute pericarditis" and in one patient serious consideration was given to the possibility of an "acute coronary episode."

SUMMARY

1. The unipolar electrocardiograms of 681 consecutive admissions to the Veterans Administration Hospital, Madison, Wisconsin, were studied for the incidence of the "juvenile pattern." Of the total of 131 adult Negro males, fourteen (10.8 per cent) were found to show persistent or transient T wave inversion patterns in the unipolar leads V₁ through V₆. All fourteen patients were thought to have normal cardiovascular systems, with no clinical evidence of pericarditis. Cases 3, 7 and 9 were noted to have normal pericardiums at time of thoractomy.

2. The "juvenile pattern" was consistently normalized by the oral administration of 10 gm. of potassium bicarbonate-citrate mixture and by the intravenous administration of 20-30 mg. of pro-banthine. Hyperventilation consistently exaggerated the T wave inversion pattern, an effect which could be abolished by the administration of pro-banthine.

3. The "juvenile pattern" is believed to represent an expression of hypervagotonia and is considered to be a normal variant of the adult Negro.

4. One must be cautious in the interpretation of these transient electrocardiogram changes in the adult Negro if one wishes to avoid erroneous diagnoses of "subepicardial myocarditis," "myocardial ischemia" and "subacute pericarditis," and the risk of promoting serious iatrogenic heart disease.

Acknowledgments: The author expresses his appreciation to Doctors O. O. Meyer, J. K.

Curtis and F. C. Larson for helpful advice and criticisms, to Mrs. J. Lloyd for technical assistance and to Mrs. F. Lee for secretarial work. The electrocardiographic reproductions were done by Mr. F. J. Fischer.

Addendum. Following the completion of this manuscript, three of thirty Negro males subsequently admitted to the Madison Veterans Administration Hospital between February and June, 1954, have satisfied the criteria for a variation of the electrocardiographic "juvenile pattern." Hyperventilation consistently produced or augmented T wave inversion in the precordial leads and all patients showed prompt normalization of the inverted precordial T waves following pro-banthine injection.

A single patient, a twenty-two year old Negro, was seen in consultation at the Lakeview Tuberculosis Sanatorium, Madison, Wisconsin. Serial electrocardiograms over a two-year period had shown persistent T wave inversion in CF₂, CF₃ and CF₄. The diagnosis of tuberculous pericarditis had been entertained. Prompt normalization of the inverted precordial T waves was effected by the administration of oral potassium salts and intravenous pro-banthine. Following a left upper lobectomy in December, 1953, serial electrocardiograms have shown considerable variability in the T wave configuration across the precordial leads. At time of thoracotomy, the pericardium was noted to be uninvolved by the tuberculous process.

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Arteriosclerotic Heart Disease in Diabetes Mellitus*

A Clinical Study of 383 Patients

IRVING M. LIEBOW, M.D., HERMAN K. HELLERSTEIN, M.D. and MAX MILLER, M.D.
Cleveland, Ohio

THERE have been many autopsy studies of arteriosclerosis of the coronary arteries in patients with diabetes. These papers were described in a previous review.¹ Studies of the prevalence of arteriosclerotic heart disease in living diabetics, however, have been less numer-

Medical Outpatient Department of the University Hospitals of Cleveland. Their hospital and outpatient charts were reviewed, with note made of all previous symptoms, physical findings, roentgenograms of the heart, electrocardiograms and blood pressure readings. The new work-up

TABLE I
CARDIAC FINDINGS IN LIVING DIABETICS

Author	Remarks	Angina Pectoris (%)	Myocardial Infarct (%)	Arterosclerosis of the Aorta (%)
Rabinowitch et al. ⁴	1,500 outpatient diabetics	1.3	0.46
Friedman ³	120 outpatient diabetics over age thirty-nine	9.0	60-75
Edeiken ⁶	100 outpatient diabetics with diabetes ten yr. or more; 80 per cent over age forty	8.0	1 certain (4 probable)	24 (all over age fifty)

ous. As recently as 1953 Lundbaek² stated in his monograph: "The literature contains only little information about cardiac symptoms and examinations of the heart in living diabetics." We have summarized the literature in Table I.

This is a preliminary report of an investigation of the prevalence of heart disease in living, diabetic outpatients. Three hundred eighty-three such patients were studied. A later communication will deal with heart disease in general and with data on hypertension and the kidney in a larger group of such patients.

METHOD

During the period 1948 to 1953, 383 patients with diabetes mellitus were chosen at random from the attenders in the Diabetes Clinic of the

for purposes of this report required two to four of the succeeding regular visits to the Diabetes Clinic. The patient was questioned again in regard to symptoms of heart disease, and a detailed physical examination was made. An electrocardiogram (bipolar and augmented unipolar limb leads, and leads V₁₋₆) and roentgenograms of the heart were obtained and the total serum cholesterol was determined. Notation was also made of age and weight, the duration of the diabetes and the insulin dose in use at the time of the physical examination. The over-all degree of control of the diabetes was also noted, taking into consideration the patient's entire recorded diabetic history up to the time of the examination.

The diagnostic criteria of the New York Heart

* From the University Hospitals of Cleveland and the Department of Medicine, School of Medicine, Western Reserve University, Cleveland, Ohio. Supported in part by a grant from the Cleveland Area Heart Society.

Association³ were employed. Thus the etiologic classification "arteriosclerosis" was limited to those cases of heart disease showing definite evidence of arteriosclerosis of the coronary arteries or the arch of the aorta. The diagnosis was based on symptoms or abnormal findings "indicating arteriosclerosis of the coronary arteries, thrombosis or occlusion of one or more coronary branches, fibrosis of the myocardium, sclerosis of a valve, (and/or) arteriosclerosis of the aorta." A diagnosis of arteriosclerotic heart disease was made on the basis of the electrocardiogram alone, only when evidence of myocardial infarction was found. Other electrocardiographic abnormalities (e.g., conduction defects, premature beats, T wave changes, S-T displacement) were considered only as confirmatory and did not in themselves constitute a diagnosis of arteriosclerotic heart disease.

All correlations were statistically analyzed by the Department of Preventive Medicine of the School of Medicine of Western Reserve University.

Data on the Group Studied. *Age:* The age distribution of the 383 patients is shown in Figure 1; 90.1 per cent of the patients were over age forty. The average age of the group was 58.1 years. The average age for the males was 59.4 years; the average age for the females was 57.7 years.

Sex: There were 282 women (73.6 per cent) and 101 men (26.4 per cent) in the group. This gives a ratio of females:males of 2.78:1.

Race: There were 188 white persons (49.1 per cent) and 195 Negroes (50.9 per cent) in the group. This gives a ratio of white:Negro of 1:1.

RESULTS

Arteriosclerotic Heart Disease. Of this group of 383 diabetic patients, 161 (42 per cent) had clinical arteriosclerotic heart disease.

Wartman and Hellerstein⁴ reported on 2,000 consecutive autopsies from this same institution (The University Hospitals of Cleveland). They found the incidence of coronary artery disease to be 23.3 per cent. This is appreciably lower than the 42 per cent found in our diabetic group, particularly when one considers that the incidence of coronary artery disease is highest in examinations at postmortem.

Comparison of our findings in a diabetic group with the prevalence of clinically diagnosed arteriosclerotic heart disease in the general

population is difficult because reports on the latter are scarce. Recently, however, Epstein and Boas⁵ found coronary artery disease in 7 per cent of 568 people over age forty. There were 343 men (mean age sixty-nine), 10 per cent of whom had evidence of coronary artery disease,

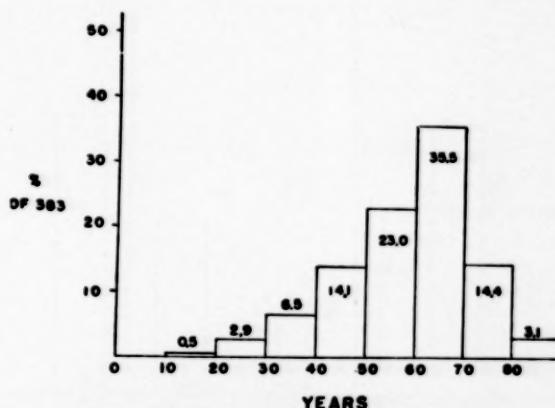


FIG. 1. The age distribution of the 383 diabetics studied. The number at the top of each column represents the per cent of the total in each age group.

and 225 women (mean age fifty-four), 2 per cent of whom had evidence of coronary artery disease. Our 161 patients with arteriosclerotic heart disease were all in the age group forty and over, which group numbered 345. Thus in our series the prevalence of arteriosclerotic heart disease was 46 per cent. This is a marked increase. It must be stated, however, that our group and that of Epstein and Boas are not strictly comparable, even excluding the factor of diabetes: theirs is an employed group (chosen at random from a union membership) which in all likelihood automatically eliminated a number of obvious cardiac patients. This employed group did include some diabetics, however. Figures on a general population will eventually be available from the Framingham study.⁶

Arteriosclerosis of the Aorta. Of this group of 383 diabetics, 161 (42 per cent) had clinical arteriosclerotic heart disease. An additional sixty-two patients (16.2 per cent) had what was formerly called "potential" heart disease, diagnosed on the basis of arteriosclerosis of the aorta as the predisposing etiologic factor. Thus 223 of the 383 patients studied (58.2 per cent) had demonstrable arteriosclerotic heart disease or evidence of the predisposing etiologic factor.

Teleradiograms of the chest showed calcium in the aorta in 126 (32.9 per cent) of the entire group. It was present in 34 men (33.7

per cent of the males) and in 92 women (32.6 per cent of the females).

Angina Pectoris. Thirty-nine patients (10.2 per cent) had angina pectoris. It was present in 10 men (9.9 per cent of all males) and in 29 women (10.3 per cent of all females). Thus the

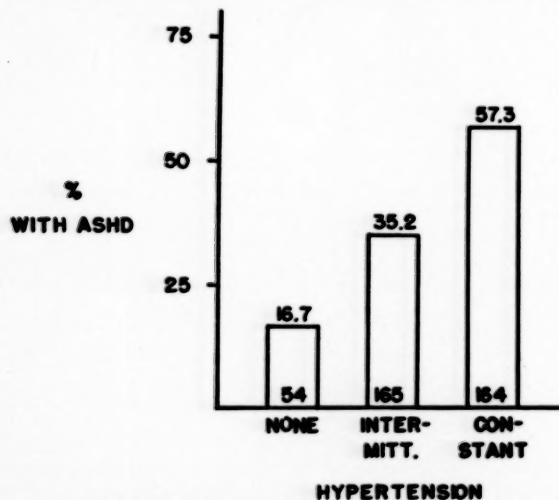


FIG. 2. The relationship of the prevalence of arteriosclerotic heart disease to hypertension in 383 diabetics. Qualification of hypertension as "none," "intermittent" and "constant" is explained in the text. The number at the base of each column represents the number of patients of the 383 diabetics in each hypertension group. The number at the top of each column represents the per cent of patients with arteriosclerotic heart disease in each hypertension group.

ratio of per cent of men with angina pectoris to per cent of women with angina pectoris is 1:1. This is in keeping with our findings concerning the sex distribution of arteriosclerotic heart disease, in general, in this group. Discussion of this point appears later.

Myocardial Infarction. Twenty-six patients (6.8 per cent) had had myocardial infarction. This had occurred in 14 men (13.9 per cent of all males) and in 12 women (4.3 per cent of all females). Thus the ratio of per cent of males to per cent of females is 3.2:1, like that of myocardial infarction in the general population. In view of the sex distribution of arteriosclerotic heart disease in general and angina pectoris in particular in this group of diabetics, and the postmortem findings in other studies, this finding in regard to myocardial infarction in our group is difficult to explain.

It is interesting to note that in four cases (one man and three women) the myocardial infarction was found only by reason of the routine electrocardiogram made during the course of

this study. In these four there was no history indicative of any acute episode. Two of these four had had previous electrocardiograms, and these had not shown evidence of infarction. The figures suggest that 1 per cent of the patients in our Diabetes Outpatient Clinic have undiagnosed myocardial infarction.

Hypertension. All blood pressure readings recorded in the patient's hospital and outpatient charts were assembled in chronologic order. A patient was considered to have no hypertension if a systolic pressure of over 140 mm. Hg or a diastolic pressure of over 90 mm. Hg had never been recorded. A patient was considered to have hypertension if there was a record of a systolic pressure of over 140 mm. Hg, or a diastolic pressure of over 90 mm. Hg, or both. Hypertension was called persistent or constant only if all the readings during the entire year preceding the patient's examination were above normal as so defined. At least three readings must have been recorded during that year. If not, then at least three successive readings over any longer period of time immediately preceding the examination must have been above normal. All other patients were considered to have intermittent or inconstant hypertension.

The relation of the prevalence of arteriosclerotic heart disease to normal blood pressure, intermittent hypertension and persistent hypertension is shown in Figure 2. The progressive increase in prevalence of arteriosclerotic heart disease from those diabetics with normal blood pressure through those with intermittent hypertension to those with constant elevation of the blood pressure is readily apparent. Statistical analysis shows that the differences noted are significant ($P = <.001$). Similar results have been evident in necropsy studies. Root and Sharkey⁷ reported that in diabetics with hypertension, coronary arteriosclerosis was two and a half times as frequent and coronary thrombosis three times as frequent as in diabetics without hypertension. Similarly, Stearns, Schlesinger and Rudy,⁸ in their study of diabetics, found that in the presence of high blood pressure both angina pectoris and death due to acute coronary disease were more common.

This positive relationship between the prevalence of arteriosclerotic heart disease and the presence of hypertension in living diabetics is of interest. Hypertension generally is associated with a higher incidence of coronary arteriosclerosis.^{9,10} Furthermore, hypertension occurs

more commonly in diabetics than in non-diabetics.^{11,12} It would seem reasonable to conclude, then, that the diabetic population must inevitably have more coronary heart disease than the non-diabetic population. That this is so has been proved often at the necropsy table; unfortunately, there are no clinical studies on the prevalence of arteriosclerotic heart disease in living non-diabetic people (i.e., the general population) with which to compare our figures.

The positive relationship in this group is of interest for another reason. The criteria for hypertension used in this study are those of the New York Heart Association: a systolic reading of over 140 mm. Hg and/or a diastolic reading of over 90 mm. Hg. In the literature this is generally the lowest level considered for a diagnosis of high blood pressure. Master *et al.*^{13,14} consider hypertension of this degree, in people over age forty, so common as to suggest that mild or even moderate elevations above this level no longer be considered abnormal. The data presented in this report (in which all cases of arteriosclerotic heart disease were in those above age forty) indicate that when patients are divided according to whether their blood pressure is over or under 140/90, significant differences are found in the prevalence of arteriosclerotic heart disease.

Cholesterol. The common occurrence of hypercholesterolemia in diabetes mellitus is well recognized. The question then arises as to the relationship of serum cholesterol level to the prevalence of atherosclerosis in diabetes. This question has been reviewed by Ricketts.¹⁵

An analysis was made of the relation of the total serum cholesterol level to the prevalence of arteriosclerotic heart disease in this group. The cholesterol level was determined in 367 of the 383 patients. The blood was drawn three to four hours after breakfast. The chemical method for determination of the total serum cholesterol was that of Kingsley and Schaffert.^{16*}

The results are shown in Figure 3. It is apparent that in this group there was no relationship between serum cholesterol level and the presence of arteriosclerotic heart disease. This is not surprising. Although various reports have shown an association between hypercholesterolemia and coronary sclerosis, the association has not always been clear-cut; distribution curves of

cholesterol levels have shown appreciable overlap between those with and those without coronary disease, and certainly no causal relationship has been proved. Indeed, Gertler *et al.*¹⁷⁻¹⁹ on the basis of their studies believe that the development of coronary arteriosclerosis is

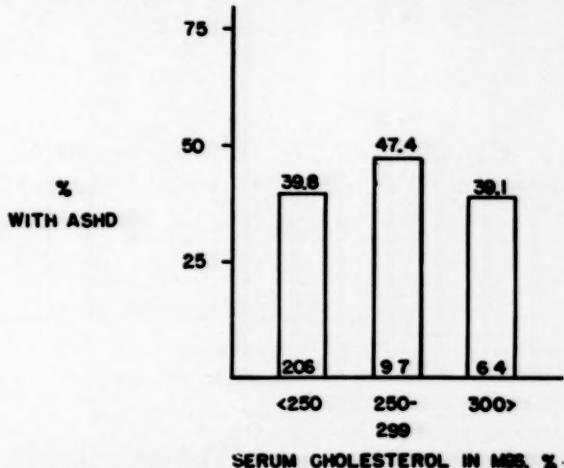


FIG. 3. The relationship of the prevalence of arteriosclerotic heart disease to the total serum cholesterol level in 367 diabetics. The number at the base of each column and the number above each column follow the arrangement explained in the legend of Figure 2.

not at all dependent on the absolute serum cholesterol level. They suggest, rather, that a "specific predisposition" (Pc) to the disease, perhaps in the form of a lipid factor, is superimposed on the normal lipid metabolism. With this factor present, coronary arteriosclerosis could develop without a high serum cholesterol level. Gofman *et al.*^{20,21} indict lipid molecules of the S, 10-20 class as the offending agent.

Control of the Diabetes. An important question has been whether or not day to day control of the diabetes decreases the incidence or delays the appearance of the vascular complications of the disease. The answer, not yet complete, has had to await the passing of time. Dolger²² examined 200 diabetics who had had the disease for twenty-five years and reported that not one escaped retinal hemorrhage, albuminuria and/or hypertension. Keiding, Root and Marble²³ studied the relationship of the control of diabetes to the development of retinopathy, arterial calcification and nephropathy. It was their opinion that excellent or good control of the diabetes materially decreased the incidence of these complications. Neither report considered the relationship of control to the occurrence of coronary arteriosclerosis.

* All determinations were made in the Biochemistry Laboratory of The University Hospitals, under the direction of Dr. Waide Price.

In this report control was determined on the basis of the patient's entire hospital history, with careful consideration given to all urinalyses made during all outpatient visits. Control was considered to be "excellent" if no glycosuria was noted at any time. It was considered to be

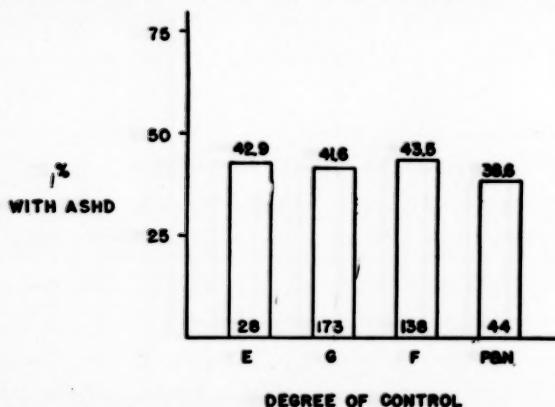


FIG. 4. The relationship of the prevalence of arteriosclerotic heart disease to the degree of control in 383 diabetics. "E," excellent control; "G," good; "F," fair; "P and N," poor and none. The numbers within and above the columns follow the pattern outlined for the preceding graphs.

"good" if the majority of the urines tested was negative for sugar, with an occasional one plus glycosuria but no ketonuria noted. A rating of "fair" was given if the urines were usually 1 to 3 plus, with no ketonuria noted. Control was labeled "poor" if the urines were 3 to 4 plus constantly; in this group were also placed those patients who had shown ketonuria and those who had episodes of acidosis. A degree of control of "none" was given in four instances. These patients all needed insulin but had not had it for a long period of time immediately preceding the examination. One patient was schizophrenic and, although generally capable of caring for herself, was not capable of administering insulin; the other three patients simply did not take insulin. Because the "none" group is so small, and because the actual degree of control was similar to that of the "poor" group, the two categories were combined for purposes of analysis.

The relationship of degree of control to the prevalence of arteriosclerotic heart disease is shown in Figure 4. It is readily apparent that the occurrence of arteriosclerotic heart disease diagnosed clinically was not related to the degree of control of the diabetes. Robinson²⁴ studied a group of fifty-four diabetics with myocardial infarction and noted that the mortality was

higher in those with poor control. More comparable with our group are the fifty diabetics studied by Thaler and Wornas.²⁵ Using angina pectoris and/or myocardial infarction as the criteria, they found that coronary artery disease began at an average age of 56.1 years in diabetics with poor control and at 65.6 years in those with good control. They concluded that good control postponed coronary artery disease. We do not attempt to answer the question of time of onset. Our data simply show that the prevalence of arteriosclerotic heart disease in the group here reported was not related to the control of the diabetes.

The data were further analyzed by breaking down each classification of "control" into ten-year age groups. No consistent difference by age group was found.

Obesity. The relationship of obesity to coronary arteriosclerosis is unsettled. Reed and Love²⁶ and Levy,²⁷ in clinical studies, found "cardiovascular-renal disease" increased in the obese. Wilens,²⁸ in a necropsy study, found a distinct relationship between obesity and coronary arteriosclerosis, particularly in the male. On the other hand, Faber and Lund,^{29,30} studying both dry weight and total cholesterol content of the aorta, found no relationship between atherosclerosis and body weight. Similarly, Garn *et al.*,³¹ in a clinical study, found that those men with a myocardial infarct in early life (less than age forty) were not more overweight as a group than were their control group.

An examination was made of the relationship of arteriosclerotic heart disease in our diabetic group to the degree of obesity of the patients at the time of physical examination. The standards for normal weight were obtained from the weight-height-age table of Barach.³² The results are shown in Figure 5. It is obvious that in this group there is no positive relationship between prevalence of arteriosclerotic heart disease and obesity. Further breakdown by sex again showed no such relationship.

Severity of Diabetes. Most authors agree that the severity of the diabetes bears no relation to the development of arteriosclerosis. Friedman³³ and Dolger²² found this to be true in regard to arteriosclerosis in general in studies of living diabetics. Keiding, Root and Marble,²³ also studying living diabetics, found that patients receiving lower doses of insulin had about the same incidence of retinopathy, peripheral arterial calcification and nephropathy as did

those in the entire group. Hart and Lisa³⁴ and Bell³⁵ came to the same conclusion from postmortem studies. Similarly, Stearns et al.⁸ found that the severity of arteriosclerosis of the coronary arteries, specifically, was not proportional to the severity of the diabetes. The same

as compared to the general population, and is completely in keeping with the postmortem findings in diabetics.

An analysis was also made of the sex difference in the prevalence of arteriosclerotic heart disease within each age group. The basic figures

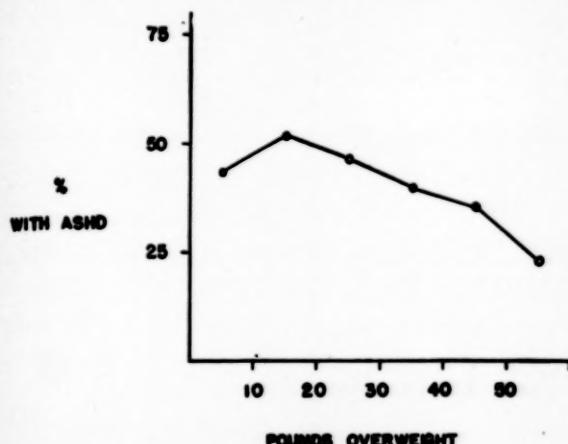


FIG. 5. The relationship of the prevalence of arteriosclerotic heart disease to pounds overweight in 383 diabetics.

conclusion is implied by Thaler and Wornas²⁵ in a study of living diabetics.

In this report the insulin requirement per day was considered as an index of the severity of the diabetes. Figure 6 shows the relationship of the daily dose of insulin to the prevalence of arteriosclerotic heart disease in the group studied. It is apparent that no positive correlation exists.

Sex. It is now generally accepted that at necropsy coronary arteriosclerosis occurs three to four times as frequently in males as in females. Similarly, the clinical manifestations (angina pectoris and myocardial infarction) occur with the same preponderance in the male. In diabetics, however, a change is noted: the known marked increase in coronary arteriosclerosis is characterized not only by an absolute but also by a marked relative increase in incidence in the female. All necropsy studies^{7,36-38} are in agreement on this point.

For this reason it was thought that it would be of interest to note the sex difference in the clinically diagnosed arteriosclerotic heart disease in this living group of diabetics. The results are seen in Table II: 47.5 per cent of the men and 40.1 per cent of the women had arteriosclerotic heart disease, giving a ratio of the per cent of men to the per cent of women of 1.2:1. This demonstrates a marked relative increase in coronary artery heart disease in the females in this group

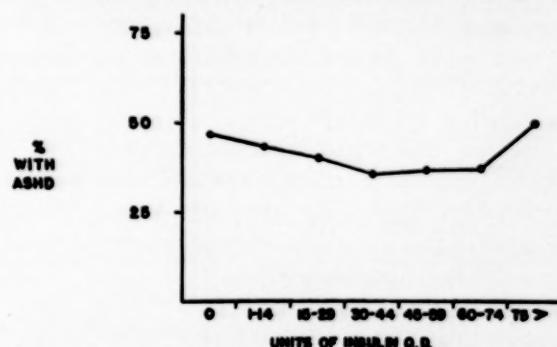


FIG. 6. The relationship of the prevalence of arteriosclerotic heart disease to the daily dose of insulin in 383 diabetics.

are presented in Table III. These data show that in diabetic patients arteriosclerotic heart disease is found with the same relative frequency in men and women in each age group.

TABLE II
PREVALENCE OF ASHD* IN 383 DIABETICS BY SEX

Sex	No.	ASHD	
		No.	(%)
Males.....	101	48	47.5
Females.....	282	113	40.1
Total.....	383	161

* In this table and those which follow, "ASHD" means "arteriosclerotic heart disease."

Age. The relation of prevalence of arteriosclerotic heart disease to age is shown in Figure 7. Below age forty there were no diabetics with clinically diagnosed coronary artery heart disease. In the decades over age thirty-nine years the prevalence of arteriosclerotic heart disease rose steadily with age to an incidence of 92 per cent in the group aged eighty and over. This is not surprising, of course. At necropsy the incidence of coronary arteriosclerosis in the general population is seen to increase with age. Likewise, in the living the prevalence of some of the clinical manifestations, such as myocardial infarction, increases with age.³⁹ For these reasons the rela-

tionship shown in our patients can hardly be solely attributed to the diabetes.

The age distribution in diabetics with arteriosclerotic heart disease is shown in Table IV: 27.3 per cent are below age sixty; 72.6 per cent are age sixty or over.

appear to accelerate the development of arteriosclerosis. Similarly, examination of Martensson's figures⁴³ shows that clinically diagnosed coronary arteriosclerosis was not related to duration of diabetes but rather to age.

Our findings are summarized in Table V.

TABLE III
ARTERIOSCLEROTIC HEART DISEASE IN 383 DIABETICS BY SEX AND AGE

Sex	Age (yr.)	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80 >	Total
Males	No. with ASHD	0	0	0	2	7	21	13	5	48
	No. in group	1	4	7	11	17	35	21	5	101
	Per cent with ASHD	0	0	0	18.2	41.2	60.6	61.9	100	...
Females	No. with ASHD	0	0	0	9	26	50	22	6	113
	No. in group	1	7	18	43	71	101	34	7	282
	Per cent with ASHD	0	0	0	20.9	36.6	49.5	64.7	85.7	...
X ²03	.11	1.12	.05	.73	...
P865	.742	.290	.826	.396	...

Duration. It has been generally recognized that the incidence of arteriosclerosis increases with the duration of the diabetes. This point has been emphasized by Warren.⁴⁰ The literature has been well summarized by Peters.⁴¹ However, the

Table VI is a condensation. Graphic representation is made in Figure 8.

Analysis of our cases shows that prevalence of arteriosclerotic heart disease is not related to the duration of diabetes. The differences, when

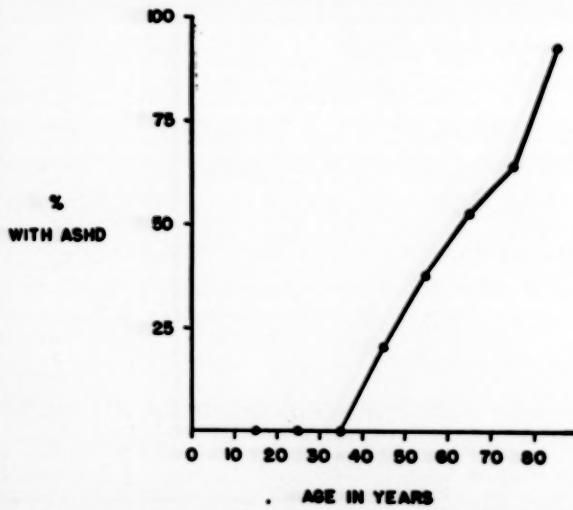


FIG. 7. The relationship of the prevalence of arteriosclerotic heart disease to the age of the patient in 383 diabetics.

published reports have consisted mostly of necropsy studies. Data on arteriosclerotic heart disease in living diabetics are scant. Boas⁴² analyzed his information on 500 living diabetics, taking into consideration arteriosclerosis of both the coronary arteries and lower extremities. He states that in his cases diabetes did not

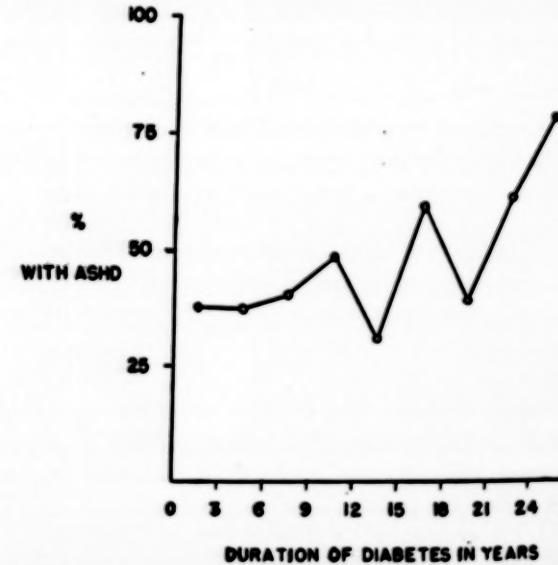


FIG. 8. The relationship of the prevalence of arteriosclerotic heart disease to the duration of diabetes in 383 diabetics.

examined by the χ^2 method, could occur by chance seven times in one hundred. Arteriosclerotic heart disease did seem more prevalent in those having diabetes more than twenty years. Since this could well be a function of age

rather than of duration of diabetes, several correlations of age to duration were made, using various age groupings. The data suggest that of those over age sixty in this group studied, the patients having diabetes more than twenty years are more likely to have arteriosclerotic

TABLE IV
AGE DISTRIBUTION OF 161 DIABETIC PATIENTS WITH
ARTERIOSCLEROTIC HEART DISEASE

Age (yr.)	Number	Per cent
Under 40	0	0
40-49	11	6.8
50-59	33	20.5
60-69	71	44.1
70-79	35	21.7
80 and over	11	6.8
Total	161	99.9

heart disease. However, statistically, the figures are not conclusive.

COMMENTS

The material presented herein is, in effect, a cross sectional view of the cardiac status of a group of diabetic patients in various stages of their diabetes. It is, however, a view of a particular group of such patients: those attending the Diabetes Clinic of the Medical Outpatient Department of The University Hospitals of Cleveland. Thus the sampling is not necessarily representative of the general diabetic population of the country, or even of Cleveland. The race distribution, for example, showed an almost even division between white and non-white. Keeping these facts in mind, we have tried to avoid drawing general conclusions from our data. Furthermore, to repeat, this is a cross sectional view—an examination of the cardiac status of the group at a given point in time. The data cannot be interpreted as giving a long-range picture of the natural development of arteriosclerotic heart disease in diabetes.

A difficulty constantly encountered in attempting to evaluate our findings was the lack of

TABLE V
NUMBER OF PATIENTS WITH ARTERIOSCLEROTIC HEART DISEASE, BY AGE GROUPS, IN RELATION TO
DURATION OF DIABETES*

Age of Patients (yr.)	Duration of Diabetes in Years									
	<3	3-5	6-8	9-11	12-14	15-17	18-20	21-23	24 >	Total
10-39	(15)	(9)	(4)	(2)	(3)	(2)	(1)	(1)	(1)	(38)
40-49	4 (21)	4 (12)	(4)	2 (8)	(4)	1 (2)	(2)	(1)	11 (54)
50-59	11 (32)	4 (13)	5 (11)	6 (14)	2 (7)	2 (2)	1 (5)	1 (3)	1 (1)	33 (88)
60-69	17 (36)	8 (16)	13 (24)	11 (17)	5 (15)	4 (6)	4 (12)	6 (7)	3 (3)	71 (136)
70-79	12 (19)	5 (6)	1 (3)	3 (6)	3 (7)	2 (3)	3 (3)	3 (5)	3 (3)	35 (55)
80 >	4 (4)	(1)	2 (2)	2 (2)	2 (2)	1 (1)	11 (12)
Total	48 (127)	21 (56)	19 (47)	24 (49)	12 (38)	9 (15)	10 (25)	10 (16)	8 (10)	161 (383)

* Number of patients in each age-duration group, with or without ASHD, is enclosed in brackets.

TABLE VI
PREVALENCE OF ARTERIOSCLEROTIC HEART DISEASE IN RELATION TO DURATION OF DIABETES IN 383
DIABETICS

Duration of Diabetes (yr.)	<3	3-5	6-8	9-11	12-14	15-17	18-20	21-23	24 >
No. with ASHD.....	48	21	19	24	12	9	10	10	8
No. in group.....	127	56	47	49	38	15	25	16	10
Per cent with ASHD.....	37.8	37.5	40.4	49	31.6	60	40	62.5	80

information in the literature concerning the prevalence of arteriosclerotic heart disease in the general, living population. The generally held belief that arteriosclerosis, including coronary arteriosclerosis, is more common in diabetics than in non-diabetics is based almost entirely on the comparison of autopsy populations. Data on arteriosclerotic heart disease in living people will probably be available from the Framingham study⁶ and from Epstein and Boas.⁵ It can be inferred from our data, however, that the prevalence of arteriosclerotic heart disease is greater in diabetics than in the general population.

SUMMARY

1. A cardiac survey was made of 383 living, outpatient diabetics. The findings in regard to clinically diagnosed arteriosclerotic heart disease in these patients are reported.

2. Forty-two per cent had arteriosclerotic heart disease. An additional 16.2 per cent had arteriosclerosis of the aorta.

3. Angina pectoris was found in 10.2 per cent; 6.8 per cent had myocardial infarction.

4. The prevalence of arteriosclerotic heart disease was related in positive manner to sex, age and the presence of hypertension.

5. The prevalence of arteriosclerotic heart disease was not related to the total serum cholesterol level, the degree of control of the diabetes, the patient's weight, the daily insulin dose or the duration of the diabetes.

Acknowledgment: The authors are most grateful to Dr. George A. Badger and to Dr. Arthur Littell, who made all of the statistical analyses involved in this report.

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Causes of Labile Diabetes: Its Treatment

JOSEPH I. GOODMAN, M.D.

Cleveland Heights, Ohio

AMONG persons with severe diabetes there exists a group referred to by Woodyatt¹ as "brittle," a term which implies that the state of control breaks easily in either direction. Other designations which have been applied to this condition by various authors are "labile," "volatile," "unstable" and "total" diabetes. Labile diabetes, the term of reference which will be employed in this paper, exhibits a characteristic pattern wherein, for no apparent reason, the patient may suddenly develop periods of heavy glycosuria and/or acidosis shifting rapidly to violent, unexpected insulin reactions. The condition appears to defy almost all attempts at regulation with orthodox treatment which controls less severe forms without difficulty. Colwell² compares the labile diabetic to a tight-rope artist in contrast to the mild diabetic who walks on a wide path.

Rapid fluctuations from glycosuria to insulin reactions are frequent occurrences in the first and second decades of life. This type of behavior is much less common among older patients with the exception of the non-obese diabetic in whom labile diabetes is quite common.

Such labile cases impose a challenge upon the physician to find the insulin type, timing and dosage which will prevent ketosis, achieve as little glycosuria as possible and, most important, permit freedom from insulin shock which, in essence, is the prime objective of diabetic therapy in general. To circumvent severe insulin reactions the clinician usually compromises between control and convenience. In other words, glycosuria is permitted as the lesser of two evils. Most authors concede that ideal control is seldom attained in this group.

The results of regulation in a series of twenty-five diabetic individuals below the age of forty years was selected from my office practice for review. Each of these patients was faithful in his office visits, was treated identically and remained under my care continuously for a minimum period of three months. The subjects in this group ranged in age from six to forty years, with

an average age of twenty-four years. (Table I.) There were twelve males and thirteen females and all were white persons. Six of the twenty-five cases were newly discovered diabetics. In the remaining nineteen patients the duration of diabetes at the time of their first visit varied from four months to twenty years, with an average

TABLE I GENERAL DATA			
Total Cases:			25
Male		12	
Female		13	
Duration of Diabetes:	19 Cases	4 mo.-20 yr.	
Average		8.7 yr.	
Age:	6 Cases	Newly found	
Average		6-40 yr.	
Period of Observation:		24 yr.	
Average		3 mo.-12 yr.	
Period Required for Regulation:		32 mo.	
Average	19 Cases	1 wk.-6 yr.	
	6 Cases	92 wk.	
		Not regulated	

of 8.7 years. All cases have been observed continuously from a minimum of three months to a maximum of twelve years, an average of thirty-two months per patient. In other words, the group as a whole was followed over 68.7 years of diabetic existence.

The frequency and severity of ketosis and hypoglycemia in these twenty-five patients while on their previous regimens are compared with the incidence of these complications while under treatment by the procedure to be described. (Table II.) The seven newly discovered cases, having had no previous therapy, can be evaluated only as to their course under our management. Eight of the remaining eighteen patients gave a previous history of having had one or more episodes of keto-acidosis, with an episode of coma in one case. Nine patients had a history of significant insulin reactions and of these five had repeated and severe reactions. Two patients had both recurrent keto-acidosis (one had coma) and severe insulin reactions. While under the treatment to be described one mild episode of ketosis (in a ten year old boy) occurred among

the twenty-five cases. Only ten of the twenty-five patients had insulin reactions of any sort and in one-half of these (five cases) the reactions occurred only at the onset, i.e., during the period of diabetic regulation. The nature of the insulin reactions varied from slight to very slight and no severe reactions have been encountered.*

TABLE II
HISTORY OF KETOSIS AND HYPOGLYCEMIA

	No. of Cases	
	Before	After
Ketosis.....	8	1
Coma.....	1	0
Major reactions.....	9	0*
Repeated reactions.....	5	0
Reactions and ketosis.....	2	0

* Minor reactions in ten cases.

A total glycosuria of 1 to 10 gm. in twenty-four hours is the standard for the regulation of diabetic patients which I have adopted arbitrarily. This ideal goal was attained in nineteen patients. To date, six patients who, after periods of ten to twenty-four months of treatment, persistently excrete more than 10 gm. of glucose in twenty-four hours have not met these criteria. However, I would stress the point that the relatively infrequent changes in insulin dosage is one prime reason for the success of the method. In those patients in whom ideal control was achieved an average period of ninety-two weeks per patient elapsed, varying from a minimum of one week to a maximum of six years (in one patient). I believe that the low incidence of hypoglycemia with this method of treatment is attributable in part to the caution exercised before altering the insulin formula which in turn necessitates a relatively long period of regulation.

Probably the most crucial factor in the favorable course of the patients treated with this regimen is the type of insulin employed and the mode of its administration. Fifteen of the seventeen well regulated patients had previously

* Since these data were analyzed a severe insulin reaction (unconsciousness) occurred in one of these twenty-five patients, a sixteen year old girl who had been under treatment for seven years without even a slight reaction. She overslept and omitted breakfast on a holiday.

taken a single dose of protamine zinc (eight cases), NPH (four cases) or a mixture of regular and protamine zinc insulin (three cases). (Table III.) By contrast, the final insulin formula in approximately one-half of these patients, (twelve cases) is a mixture of regular and protamine zinc insulin (usually a small dose of PZI)

TABLE III
INSULIN AND INSULIN COMBINATIONS BEFORE AND AFTER
THE PERIOD OF OBSERVATION

	Before	After
Regular insulin.....	2	7
PZI insulin.....	8	0
PZI and regular insulin.....	3	3
NPH insulin.....	4	1
PZI and regular insulin, with regular insulin in the evening.....	0	12
NPH and regular insulin, with regular insulin in the evening.....	0	1

before breakfast and a second injection of regular insulin before supper. This insulin combination proved to be necessary in only the most severe cases. Regular insulin, given in two doses (before breakfast and supper), is the second most common type of insulin program and was employed in seven cases. Three patients are maintained satisfactorily with a mixture of regular insulin and PZI administered before breakfast. The significance of these data will be discussed.

CAUSES OF LABILE DIABETES

The factors responsible for the instability of the labile diabetic are still unknown. Carelessness of the patient is frequently blamed for his poor diabetic control but more often the clinician is faced with an apparently spontaneous onset of glycosuria and ketosis or an equally unexplained episode of hypoglycemia. It is well established that infection, trauma, surgery, variations in exercise, hyperthyroidism, myocardial infarction, etc., affect the normal carbohydrate balance. However, the large majority of the metabolic fluctuations of the labile diabetic occur in the absence of any of these factors. Moreover, this behavior appears to operate in the face of constant conditions of diet, insulin and exercise. The fact that the labile cases are frequently persons with long-standing diabetes mellitus may be a clue. Some authors maintain that most children and many young adults develop lability only after long

persistence (ten to twenty years) of the disease, especially if control has not been satisfactory.

I would stress the point that approximately one-third of all patients needing insulin have severe diabetes. Sensitivity to insulin is typical of this class of diabetic patients and many of them are young. As a rule, in diabetes of such severity excessively high fasting levels are common and omission of insulin leads to severe acidosis within a few days. Too often the size of the daily insulin dose is used as a measure of the severity of diabetes. The insulin requirement may not be large despite other indications of severe diabetes and, occasionally, patients who are very sensitive to insulin need as little as 10 units daily. Actually a high insulin dosage may be more indicative of insulin resistance. Be that as it may, severe insulin-sensitive diabetics are prone to react more readily and violently to slight changes in insulin dosage.

Somogyi³ attributes the wide fluctuation in the blood sugar level in severe diabetics directly to insulin effect. He has pointed out repeatedly that hypoglycemia caused by overdoses of insulin entails a compensatory hyperglycemia and glycosuria. In other words, the hypoglycemia effects a shift in balance between glycogenolytic and glycogenetic factors. As a consequence, particularly in insulin-sensitive cases, the pendulum swings rapidly from the hypoglycemic to the hyperglycemic side. Paradoxically, the urine of patients showing marked hypoglycemia may actually contain large amounts of sugar and even ketone bodies. In these instances glycosuria may be misleading and one finds the clinical manifestations more indicative of the underlying condition.

Himsworth⁴ suggests that labile diabetes is a direct result of the imposition on clinical medicine of "chemical standards of normality" with disregard for the patient. In other words, many good physicians insist on complete "chemical" control, that is, absolutely physiologic blood sugar levels, at all costs. When absolute aglycosuria and restriction of the blood sugar level to physiologic limits are elevated to the status of objectives of treatment, treatment of diabetes must logically be directed to those ends even at the expense of hypoglycemic attacks. One is forced to agree with Himsworth that a harmful hypoglycemia resulting from attempting to satisfy chemical criteria is the antithesis of rational therapy.

Thus it would appear that while severe dia-

betic patients cannot survive without insulin, neither do they always live normally with it. Although insulin is a potent weapon against the ravages of diabetes, one must keep in mind that it is a double-edged sword. Lest the treatment become worse than the disease, avoidance of hypoglycemia is the physician's chief responsibility.

An important factor pertinent to the hypoglycemia of labile diabetes lies in the inherent properties of slow-acting insulins. In a previous study⁵ it was pointed out that disturbing insulin reactions are frequent occurrences with the protamine group of insulins. The high incidence of prolonged, violent and demoralizing hypoglycemic reactions in patients receiving protamine, or mixtures of protamine and regular insulin, further attests to the validity of these conclusions.^{5,6}

TREATMENT OF LABILE DIABETES MELLITUS

In Colwell's opinion² a fairly quick-acting insulin modification used twice daily enables one to effect a good compromise between pinpoint chemical control and convenience in the treatment of labile diabetes and can be accomplished with any of the following preparations: a mixture of three or four parts regular insulin and one part of protamine zinc insulin, globin with regular insulin added or NPH insulin with additional regular insulin. The method specifies that the total daily insulin dose be divided in such a manner that the larger dose (from two-thirds to three-fourths of the total) is taken before breakfast and the balance (from one-third to one-fourth of the total) before supper. Regular meals are somewhat smaller than average; interval feedings are given in the mid-morning, mid-afternoon and at bedtime.

Jackson and McIntosh,⁷ in their juvenile diabetics, advocate administration of regular insulin before breakfast and lunch and globin insulin before the evening meal. Mosenthal⁸ reports good results from simultaneous morning injections of separate doses of protamine and globin insulin, the latter in the smaller dosage. Others have claimed satisfactory results by giving about one-half of the total insulin dosage in the form of protamine zinc in the morning and the other half in the form of regular insulin, about equally divided between the morning and evening meals. While each of these modifications probably results in smoother control than

when the entire dose is given in the morning, none of these methods, with the probable exception of Jackson's, insures adequate control with freedom from hypoglycemia for the brittle diabetic patient. In order to avoid severe insulin reactions dosages are customarily reduced and excessive glycosuria accepted.

Undoubtedly the most effective regulation of the labile diabetic case is attained by the injection of regular insulin three or four times daily.^{1,6,7} According to Colwell, four doses of regular insulin of equal size given at approximately six-hour intervals, along with meals of equal glucose value, provides the best possible control and the least danger of insulin reactions even in the most difficult patients. An alternate somewhat more convenient method which has been proposed involves the injection of about four-sevenths of the total daily regular insulin before breakfast, two-sevenths before the evening meal and one-seventh at 3:00 A.M. The fact that it is possible to regulate severe diabetics with regular insulin^{6,9} should be ample proof that the lability, or brittleness, of these patients is due to the peculiarities of the actions of the depot (protamine) insulins rather than to uncommon endogenous factors or irregular food supplies.

A method which involves more than two insulin injections daily and disturbs sleep is not likely to be tolerated by most patients except for short periods of time. Undoubtedly this was an important factor in the development of the depot insulins. Still, one cannot gainsay the facts that (1) despite the inconveniences of multiple injections the majority of true labile diabetic patients can be regulated satisfactorily with regular insulin, and (2) regular insulin is the safest and most dependable of all the insulins.

Colwell¹⁰ maintains that glycosuria can be limited to some 20 gm. by one of the methods outlined above. Those who insist upon a single injection of one of the depot insulins undoubtedly countenance far greater amounts of glycosuria in preference to severe insulin reactions. Joslin et al.,¹¹ for example, consider a juvenile diabetic satisfactorily controlled who excretes less than 20 gm. of glucose for every 100 gm. of carbohydrate ingested, which may amount to 60 gm. or more in twenty-four hours.

In principle, insulin should be administered in as few doses daily as possible without permitting glycosuria or hypoglycemia. The goal of treatment in my cases is a twenty-four-hour excretion of not more than 10 gm. of glucose.¹² Of the

utmost importance, in my opinion, is the use of quantitative estimations of glucose in fractional urine specimens, in preference to the more conventional blood sugar determinations, as a guide to the type and dosage of insulin. Urine specimens are collected in four periods: (1) breakfast to lunch, (2) lunch to dinner, (3) dinner to bedtime and (4) bedtime to breakfast. The total glycosuria in each period is estimated quantitatively by the Somogyi method.¹³ The glucose excretion in each period is the sole basis of prescription of the appropriate type and dosage of insulin required to attain the desired limits. Every diabetic patient seems to show a distinctive pattern of glycosuria, as reflected in the fractional urines, and requires individual study to determine the most suitable insulin regimen. Not only is a small amount of glycosuria preferable to the risk of hypoglycemic reactions but constitutes a protection against them. By permitting a little sugar in the urine (10 gm. or less for a twenty-four-hour period) hypoglycemia is largely prevented.

An attempt to manage severe diabetics with one injection of protamine or any other insulin a day is usually unsuccessful because of severe hypoglycemia. If two injections are required to accomplish this end, the results are worth the additional inconvenience. There is no valid objection to two injections a day. The abandonment of protamine zinc insulin need not detract from its value in a mixture with regular insulin. In selected patients regular and protamine zinc insulin may be mixed to produce a smooth, constant insulin effect throughout the twenty-four-hour period without insulin reactions. For example, half the patients in this report take a mixture of regular and protamine zinc in the morning and a second dose of regular insulin before supper, the latter effectively controlling excess glycosuria after the evening meal.

Diet. The diabetic's diet is utilized efficiently if it is spread out as much as possible through the twenty-four hours. Experienced physicians understand that the daily food intake may require redistribution in order to avoid insulin reactions. A portion of food given prophylactically is more effective than an equal quantity when a reaction is well established. Applying this principle, Joslin¹⁴ often prescribes a small amount of carbohydrate every hour on the hour between meals (3, 4 or 5 gm.) such as found in various biscuits, until patients gain confidence and learn how the diet, insulin and exercise interact.

Hypoglycemic reactions can be largely prevented by appropriate timing of the food to conform to the time of maximum activity of the insulin one is taking. By dividing one or more meals into two parts it is possible to increase the insulin dose to larger amounts than would be feasible otherwise without provoking hypoglycemia. Thus a patient taking regular insulin receives a second feeding two hours after the first and the succeeding meal (lunch) two hours later (four to five hours after the injection). It is essential for a patient who is receiving protamine zinc insulin (in any form) to take a bedtime feeding as protection against nocturnal and early morning hypoglycemia. With mixtures of regular insulin and protamine zinc, breakfast is subdivided into two approximately equal halves, the second portion being taken two hours after the first. This satisfies the action of the regular insulin in the mixture. A portion of food, subtracted from lunch, should be reserved for a bedtime feeding. The hypoglycemic period for globin and NPH insulin is in the late afternoon, usually between 3:00 and 4:30 P.M. Reactions with either of these insulins can be avoided by eating a portion of food, which is subtracted from breakfast and taken six to seven hours after administration of the morning insulin dose. In order to extend the hyperglycemic effect of the feedings as much as possible, some protein and/or fat should be included.

Individuals who perform more physical labor on certain days should increase their food intake accordingly. The diabetic who engages in pre-meditated exercise such as dancing, skating or any other vigorous physical activity should be fortified against impending reactions by a protein feeding, for example, a meat, egg or cheese sandwich. Diabetics should carry a package of hard candy at all times to tide them over in case of unpremeditated exercise or an unavoidably delayed meal. The timing of feedings, the content of the feeding, and the relationship of feedings to the maximum activity of insulin are as important for the prevention of reactions as the dose of insulin.

SUMMARY

A series of twenty-five young diabetic patients from private practice, ranging in age from six to forty years, was reviewed for evidence of lability. During a total observation period of 68.7 years (approximately three years per patient) there

was one mild episode of acidosis in this group and no severe insulin reactions occurred. Previously, eight of these patients had suffered one or more bouts of keto-acidosis and nine patients had had significant insulin reactions.

Ideal control (less than 10 gm. of glucose exerted in twenty-four hours) was ultimately obtained in nineteen of the twenty-five patients. The average length of time required to regulate the patients in this group was ninety-two weeks per patient. After the early stage of therapy, changes in the insulin dose and formula are made cautiously, a practice which partially explains the low incidence of hypoglycemia.

Fluctuations in the clinical status of these diabetics are not inevitable but are frequently induced by long-acting and intermediate insulins. As a general rule, diabetic patients of this severity are best regulated by means of two injections of regular insulin, uncombined or in a mixture with protamine zinc, NPH or globin insulin.

Addendum: Since this paper was submitted for publication several seemingly well regulated patients reported herein, taking mixtures of insulin and protamine zinc insulin, complained of mild discomfort. Further scrutiny showed a tendency to mild hypoglycemia necessitating interval or extra feedings at times. In view of previous studies which revealed a low incidence of hypoglycemia with globin insulin, these patients were taken off the mixtures and switched to regular insulin given in the morning before breakfast and globin insulin before dinner. On this regimen the hypoglycemic symptoms disappeared immediately and they felt very well. Moreover, the total insulin dosage required to maintain good diabetic regulation was much lower than that required with the extemporaneous mixture of regular and protamine zinc insulin which they had been using.

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Blood Volume and Extracellular Fluid Volume during Administration of ACTH and Cortisone*

MACKENZIE WALSER, M.D., † DONALD W. SELDIN, M.D. and CHARLES H. BURNETT, M.D. ‡
Dallas, Texas

EDEMA is generally considered to represent an increase in extracellular fluid volume without any necessary alteration of intracellular fluid volume. The partition of the increment in extracellular fluid between its two component parts, interstitial fluid and plasma, has not been precisely defined. Although the major portion of any increase in the volume of the extracellular fluid is found in the interstitial compartment, blood volume is also increased under certain circumstances. It is not clear whether this latter change is an inevitable consequence of augmentation of extracellular fluid volume or an independent phenomenon. For example, the hypervolemia which may occur in congestive heart failure could be a direct consequence of retention of salt and water or, on the other hand, might arise from purely circulatory factors such as the increased volume of blood in the dilated heart.¹

The question of whether hypervolemia is a direct consequence of salt and water retention also bears upon the regulation of interstitial fluid volume in normal subjects. If it is a direct consequence, renal sodium excretion may be attuned to the volume of the blood;² if not, other mechanisms must be sought to explain the renal response to interstitial fluid volume expansion.³

Extracellular fluid can be increased in normal subjects by oral or intravenous salt loading, or by administering hormones which promote salt retention. The latter method is advantageous in that it produces a gradual isotonic expansion. In addition to their capacity to promote the renal retention of exogenous salt, adrenocortical hormones have been reported to expand the

extracellular fluid with sodium chloride and water derived from body cells in the absence of dietary salt.⁴⁻⁸

This paper reports observations of the effects of ACTH and cortisone on blood volume and extracellular fluid volume in man. Since only minor changes in either fluid space occurred during hormone administration when salt was restricted, the alterations produced when dietary salt was supplied are concluded to be solely those of increased salt and water content of the body. Blood volume did not increase despite considerable expansion of the interstitial fluid.

MATERIAL AND METHODS

The subjects were patients suffering from rheumatoid arthritis. Renal and cardiac function was normal, as evidenced in each subject by urinalysis, blood urea, inulin clearance, § PAH clearance § and physical and x-ray examination of the heart. None had hypertension.

During the studies each subject was kept on a constant diet containing sufficient calories to maintain body weight approximately constant. All foods were analyzed for sodium and chloride and individual food portions were weighed. Since the subjects usually consumed all of the diet, correction of the sodium and chloride intake for food portions rejected was seldom necessary. A constant daily ration of distilled water of from 1,500 to 2,200 cc. was provided for each subject. All urine, and in some cases stools, were collected and analyzed by twenty-four-hour periods. The subjects were weighed daily before break-

* From the Department of Medicine, Southwestern Medical School of the University of Texas, and the Medical Service, City-County Hospital, Dallas, Tex.

† Present address: National Heart Institute, Bethesda, Maryland.

‡ Present address: University of North Carolina School of Medicine, Chapel Hill, North Carolina.

fast at the end of each period. Sodium and chloride balances reported herein are calculated as the difference between measured intake and measured output. No correction has been applied for losses through the skin except in subject J. M. in whom a daily loss of 6 mM. through skin and fecal excretion has been assumed during a period of normal salt intake. Since the subjects were in an air-conditioned ward, losses through the skin were probably minimal.

The drugs were administered by intramuscular injection, every six hours in the case of ACTH and every eight hours in the case of cortisone.

Plasma volume was measured by T-1824⁹ using a single ten-minute sample. In one subject red cell mass was measured by P³²-labelled erythrocytes, using the method of Reid and Orr.¹⁰ Hematocrits were performed in quadruplicate in capillary tubes, on blood collected anaerobically and defibrinated with mercury. No corrections for trapped plasma or the difference between venous hematocrit and body hematocrit were applied.

Extracellular fluid volume was measured with S³⁵ labelled sulfate and, in one subject, with inulin. In the radiosulfate method the difference between a sample of serum obtained eighteen minutes after injection of 100 microcuries of S³⁵O₄ and a control sample was compared to the injected material, assuming that 4 per cent of the dose was excreted during this time.¹¹ Radioactivity of serum and diluted injection material was measured directly in aluminum-foil-covered stainless steel cups, using a flow counter.¹² Serum values* were corrected for density,¹² water content of 93 per cent, and an assumed Donnan factor for sulfate of 0.90.¹³

When inulin was used, urine was collected sixteen to nineteen hours following a priming injection of inulin and a constant infusion for five to six hours, using a constant infusion pump.¹⁴ Serum inulin concentrations were corrected for a water content of 93 per cent.

Venous pressure was measured with a conventional water manometer, using a vein in the right antecubital fossa. The subject was recumbent on a table and fasting and rested for fifteen minutes prior to the determination. Serum proteins were determined by the biuret method.¹⁵

Sodium was determined by means of a Barclay flame photometer, using an internal standard. Chloride was determined according to Peters

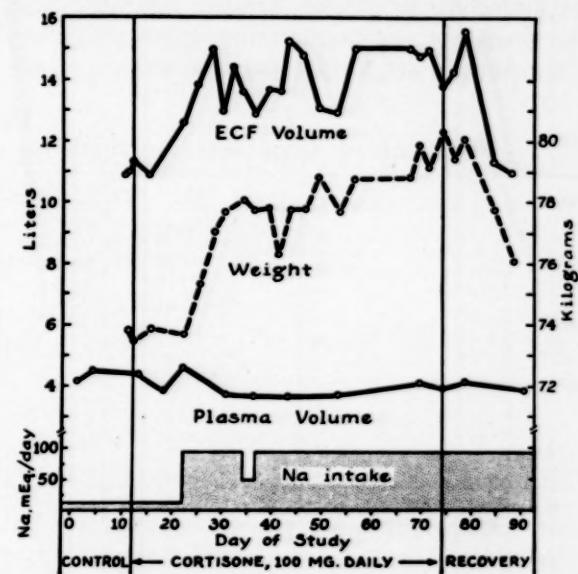


FIG. 1. The changes in weight, extracellular fluid volume (radiosulfate space) and plasma volume (T-1824) during administration of cortisone. Note that the control measurements of both volumes are consistent, and that the rise in ECF precedes the increase in sodium intake and gain in weight. The excess of final weight over control weight indicates a gain of cell substance or fat.

and Van Slyke.¹⁶ Inulin was determined according to Roe¹⁷ and PAH according to Smith.¹⁸

RESULTS

1. *Changes in Body Fluids during Salt Restriction and Hormone Administration. (Table 1.)* In five subjects the effects of ACTH and cortisone on blood volume extracellular fluid volume were determined while the patients were maintained on a low salt diet containing 10 to 12 mM. of sodium chloride daily. The cumulative balance of sodium and chloride from the first day of therapy has been noted on days when a space measurement was made. As already mentioned, these balances include no correction for skin losses in any subject, and fecal electrolyte excretion was determined in only two of the five subjects. These figures therefore, represent the maximum possible retention of electrolyte, and

$$\begin{aligned} \text{* Thus ECF volume} &= \frac{\text{Counts injected} - 0.04 \text{ (counts injected)}}{(\text{Serum counts/cc.} - \text{control counts/cc.})} \times 1.032/0.93 \times 0.90 \\ &= 0.779 \times \frac{\text{Counts injected}}{\text{Serum counts/cc.} - \text{control counts/cc.}} \end{aligned}$$

(The factor 1.032 is the density correction for normal serum relative to water.¹²)

must actually be in excess of the true cumulative balance, particularly in the longer studies. During the control periods daily electrolyte balances became zero or slightly positive after the first few days. Since the cumulative balances during these periods are simply an approximate meas-

indicated a slight but definite increase during therapy. These increments appear to be greater than could be accounted for by sodium and chloride retention, when unmeasured fecal losses in three of the five subjects and minimal losses through the skin¹⁹ are taken into consideration.

TABLE I
EFFECT OF ACTH AND CORTISONE DURING SALT RESTRICTION ON BODY FLUIDS*

Sub- ject	Period	Day	Serum Concen- tration (mEq./L.)		Maxi- mum External Balance (mEq.)	Compartments of Body Fluids				Weight (kg.)
			Na	Cl		Na	Cl	Plasma Vol. (ml.)	Red Cell Vol. (ml.)	
J. T.	Control, 12 days	1	137.0	98.0	4,120	2,700	6,820	74.03
		4	138.6	99.2	4,480	2,970	7,450	74.09
		11	4,350	2,770	7,060	10.9 11.0	73.81
	12	135.6	96.0	11.3	73.45
	Cortisone, 100 mg./day	3	141.6	97.2	+14 +11	10.9	73.32
		6	144.2	100.8	+22 +20	3,770	2,510	6,280	73.02
		10	142.1	101.2	+30 0	4,590	3,070	7,660	12.6	73.58
D. V.	Control, 4 days	1	139.2	106.4	8.9	70.45
		4	144.7	106.8	69.45
	Cortisone, 250 mg./day	1	142.7	105.6	- 2 - 7	8.5	69.08
		3	143.3	102.4	-20 -20	9.4	68.90
		6	141.0	101.2	+13 -18	9.6	68.83
		8	143.4	100.0	+35 - 9	10.0	69.07
S. S.	Control, 6 days	4	146.1	105.2	2,560	2,320	4,880	7.6	56.46
		6	144.0	101.6	2,430	2,190	4,620	7.3	56.43
	Cortisone, 250 mg., 4 days 125 mg., 3 days	4	145.5	101.6	+45 +15	2,880	2,340	5,220	8.3	56.39
G. F.	Control, 10 days	7	143.5	100.0	+77 + 2	2,780	2,230	5,010	9.6	56.52
		9	5.2	42.27
	Cortisone, 300 mg. 1 day 200 mg., 1 day 100 mg., 10 days	6	0	5.8
		9	-23	5.3
		12	-65	5.9
		16	-90	6.2

* Balance data are cumulative from the start of therapy, measured as difference between measured intake and output, which includes fecal losses in subjects J. T. and D. V. Italicized figures for cell volume are calculated by difference.

ure of the amount of salt lost in response to dietary salt restriction, they are not included in the table.

The blood volume determinations showed little change. Extracellular fluid determinations

For example, in subject D. V. chloride balance during eight days of cortisone therapy was negative and sodium balance only slightly positive. The calculated change in "chloride space" during eight days of drug administration

is plus 0.3 L. and the calculated change in "sodium space" is plus 0.2 L. Yet the measured change in extracellular fluid volume was plus 1.5 L. (The control observation of ECF in this subject, 8.9 L., was obtained at the onset of salt restriction; the ECF at the start of therapy

essentially salt-free diet. The reason for the difference between these results with inulin and our results with radiosulfate is not apparent. It could be ascribed to greater permeation of radiosulfate than inulin into the extracellular fluid before therapy and equal permeation

TABLE II
EFFECTS OF ACTH WITH AND WITHOUT SALT ON INTERNAL EXCHANGES AND URINARY EXCRETION
OF SALT AND WATER*

Period	Duration (days)	ACTH (mg. day)	NaCl (mM. day)	Day	Serum Concentrations		VP (mm. H ₂ O)	Serum Protein		External Balance		Compartments of Body Fluids				Weight (kg.)
					Na (mEq./L.)	Cl (mEq./L.)		Total (gm. %)	Alb. (gm. %)	Na (mEq.)	Cl (mEq.)	Inulin Space (L.)	Plasma Volume (ml.)	Red Cell Volume (ml.)	Blood Volume (ml.)	
I	17	0	11	10	139.0	100.0	56	6.4	4.0	48.15
				16	139.3	104.4	69	6.1	3.8	2,530	1,820	4,350	47.95
				17	2,450	1,950	4,400	48.30
II	14	250	11	6	141.2	103.2	63	6.1	3.8	+ 67	+ 41	2,560	1,660	4,220	48.12
				14	140.5	98.0	68	5.6	4.0	+152	+101	2,950	1,590	4,540	47.43
III	11	0	11	6	136.5	106.4	50	5.5	3.7	-233	-38	46.37
				11	137.9	105.2	66	6.4	4.1	-280	-94	45.66
IV	20	0	175	9	139.7	108.8	+447	+347	8.7	47.54
				19	142.9	98.8	58	5.4	3.6	+385	+304	2,950	1,530	4,480	47.36
V	20	250	175	3	143.5	109.2	..	5.2	3.6	+457	+318	49.46
				8	145.9	101.6	64	+545	+184	48.93
				10	144.8	99.2	85	5.8	3.7	+563	+221	2,860	1,440	4,300	48.72
				12	142.7	102.0	80	5.8	4.1	+680	+330	49.68
				14	143.7	100.0	67	6.0	..	+683	+330	49.64
				18	148.1	93.2	66	+735	+31	2,690	1,320	4,010	47.73
				20†	140.6	99.6	..	5.5	4.0	+743	+310	12.4	48.73

* Balance data calculated cumulatively for each period, as difference between daily intake and urine output, minus 6 mEq. during the periods of higher salt intake (allowance for stool and skin losses, subject J. M.).

† Calculated by difference.

‡ Received 400 mM. KCl p.o. on days 19 and 20.

probably was about 1 L. less, as evidenced by the weight loss of 1 kg. After one day of therapy it was 8.5 L.) Thus the greatest retention of sodium and chloride which could have occurred is insufficient to account for the observed expansion of the radiosulfate space.

Similar calculations may be made for subjects J. T. and S. S. In subject G. F. serum chloride determinations were not made so that "chloride space" change cannot be calculated. However, the loss of 90 mEq. of chloride in the face of 1 L. expansion of ECF is convincing evidence that a similar phenomenon occurred in this subject.

The magnitude of the increments of fluid added to the ECF from internal sources in these subjects is much less than that reported by Levitt and Bader.⁴ They found increases of as much as 40 per cent in the inulin space several days after giving ACTH or cortisone to patients on an

afterward. However, the radiosulfate space is, on the average, equal to the simultaneously determined inulin space.^{11a, 11b}

2. Changes in Body Fluids during Salt Administration with Hormones. In one subject (J. T.) studied at length, twenty-six determinations of extracellular fluid volume ($S^{35}O_4$)^{*} and thirteen determinations of plasma volume (T-1824) were made. Following a control period, cortisone

* This should not constitute a radiation hazard. Since 89 to 95 per cent of the radioactivity is excreted in two or three days,^{12, 30, 31} the total amount remaining in the body after the last determination in this subject cannot have exceeded 100 microcuries. If this were entirely retained in the body and eventually decayed there, it would produce only 0.6 roentgen equivalents physical during the first week and progressively less thereafter. No effects were noted following a single injection of 2,900 microcuries of S^{35} as sulfate in a human subject by Gottschalk and Allen.³⁰

(100 mg. daily) was administered for ten days with 12 mM. dietary sodium chloride and then for fifty-two days with 90 mM. dietary sodium chloride. The results during the low salt period are presented in Table I. On the higher salt intake a large positive balance of sodium and chloride and a weight gain of several kg. was noted. (Fig. 1.) Weight has been included in the figure on the days on which extracellular fluid volume was determined. Edema appeared on the fifth day and persisted in varying degree throughout the period of drug administration. An increase of 3 to 5 L. in the extracellular fluid was noted but plasma volume remained constant. The fluctuations in extracellular fluid volume in this subject are in part to be attributed to the administration of various potassium salts. These findings, as well as the electrolyte balances, are to be reported in detail elsewhere.²²

During the recovery period ECF volume fell to the control level and plasma volume did not change. The final weight was 2 kg. in excess of the control weight, which probably indicates a gain of intracellular fluid, protein or fat.

Two main points are to be noted in this study: (1) Plasma volume remained constant although interstitial volume increased as much as 60 per cent; (2) plasma volume did not reflect day-to-day fluctuation in the degree of expansion of the interstitial fluid space.

In subject J. M., ACTH was administered in two periods, first with a low salt intake (11 mM.) and then with 175 mM. (10.2 gm.) daily. The diet remained constant. Blood volume was measured with P^{32} -labelled erythrocytes and extracellular fluid volume on two occasions with inulin. The results are shown in Table II. This subject was slightly anemic (control hematocrit 34.2 per cent) but since red cell mass did not increase during therapy interpretation of the blood volume measurements is not obscured by the coincident correction of anemia.

Despite large positive balances of sodium and chloride and an increase in inulin space from 8.7 to 12.4 L., blood volume did not increase, in fact apparently decreased somewhat, although this change (12 per cent) is of doubtful significance.* Peripheral venous pressure rose very slightly but serum protein concentrations did not change appreciably. The failure of the recorded weights to reflect the gain in extracellular fluid indicates a concomitant loss of cell fluid and

* The fall in red cell mass was probably due in part to the removal of blood samples.

protein, which was borne out by a negative nitrogen balance during this period.

COMMENTS

These studies show that increase of blood volume is not a necessary sequel to salt and water retention nor an inevitable consequence of adrenocortical steroid administration. In two of the three subjects in whom salt was restricted the plasma volume rose slightly during therapy, while in the two subjects in whom salt was administered plasma volume remained constant or fell slightly. Since the only difference between these groups was the salt content of the diet, and the former group included both of the subjects of the latter group who served as their own controls, we may conclude that salt and water retention, at least under these conditions, need not in itself lead to an elevation of blood volume.

Several other reports have indicated that plasma volume expansion, usually slight, may occur during adrenal hormone administration in non-anemic subjects although in some cases a fall in plasma volume was observed.²³⁻³¹ Desoxycorticosterone has been studied more extensively in this connection, and the tendency to hypervolemia with this hormone evidently exceeds that with either whole cortical extract or cortisone.^{23,29,31-35} Cortical extract and cortisone have apparently not been shown to expand blood volume above normal in animals, although they share with desoxycorticosterone the ability to correct or prevent hypovolemia following adrenalectomy.^{7,8,32,34,36,37}

In considering the significance of these findings with respect to the general question of interstitial fluid volume regulation, it is helpful to separate those causes of edema which represent primary alterations in the Starling forces with secondary retention of salt and water, and those which are characterized by salt and water retention primarily, with or without secondary changes in the Starling forces. In the former type of disturbance it is to be expected that the blood volume is initially reduced, since the increment in interstitial fluid is initially derived from the plasma. In the latter type, however, there is no *a priori* reason for predicting an alteration of the blood volume. However, if it can be shown that in this latter type of edema the blood volume is, in fact, expanded, one may expect to observe a compensatory rise in blood volume in the former type of edema as it progresses. In such a scheme

the renal excretion of salt and water is seen as directly responsive to the blood volume, in both directions.

On the other hand, if blood volume expansion is not regularly seen in the edema which arises from salt loading in subjects without a disequilibrium of Starling forces, one is led to the conclusion that the renal excretion of excesses of salt and water in normal individuals is not primarily conditioned by receptors responsive to the volume of the blood.

The causes of edema which can be ascribed to a disequilibrium of Starling forces are somewhat uncertain; hypoproteinemia would appear to be the chief example. In nutritional edema with hypoproteinemia the plasma volume is reduced and rises during recovery.³⁸

The types of edema in which changes in the Starling forces are secondary to salt retention are (1) hormone-induced salt retention, (2) oral or intravenous salt loading in normal subjects and (3) administration of saline solution to nephrectomized animals. The results of studies of hormone-induced salt retention in addition to the present study have already been cited. Large doses of salt given orally (25 to 30 gm. daily) usually increase plasma volume whether DOCA is given concomitantly³⁹ or not.^{40,41} Reports of the effects of intravenous saline administration on blood volume in man⁴²⁻⁴⁶ are conflicting. Increases in plasma volume vary from as little as 5 per cent to as much as 50 per cent. These measurements were made from one-half hour to three hours after the end of rapid infusions, and it is likely that the results were determined largely by differences in the rate of passage of the salt solution out of the blood stream. In animals measurement of plasma volume several hours after intravenous saline solution have generally shown no increase⁴⁶⁻⁵² even after nephrectomy.⁵³⁻⁵⁶ In summary, it appears that salt and water retention does not regularly lead to sustained hypervolemia except under the special circumstances of DOCA administration or ingestion of large quantities of salt.

The failure of these observations to implicate blood volume, venous pressure or plasma oncotic pressure in the renal response to an excess of extracellular fluid leaves little room for speculation as to the possible mechanism of this response. Evidently the adrenal cortex is not necessary for its operation since, in untreated Addison's disease, salt balance can be maintained on a wide range of salt intakes.⁵⁶

SUMMARY

The effects of cortisone and ACTH on blood and extracellular fluid volumes have been studied in normotensive subjects without renal or cardiac disease, under conditions of (1) salt restriction and (2) controlled salt intake.

When salt was restricted, blood volume did not change significantly but the volume of fluid available for dilution of radiosulfate increased slightly, the increment being derived from internal sources.

When salt was supplied, extracellular fluid expanded considerably but venous pressure and plasma protein concentration did not change significantly. Blood and plasma volume remained constant or fell slightly as extracellular fluid volume expanded, and failed to reflect day-to-day fluctuations in the interstitial space.

The stimulus which promotes renal adjustment to an excess of interstitial fluid apparently need not involve blood volume, venous pressure or plasma oncotic pressure.

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Abnormal Serum and Urine Proteins in Thirty-five Cases of Multiple Myeloma, as Studied by Filter Paper Electrophoresis*

ELLIOTT F. OSSERMAN, M.D. and DOLORES P. LAWLER, B.A.

New York, New York

THE rapidly expanding literature on the methodology and applications of zone electrophoresis attests to the wide range of usefulness of this principle. The use of filter paper strips or sheets, saturated with buffer solution, as the medium for the electrophoretic separation of charged molecules, particularly proteins, was developed independently by a number of investigators in the period from 1948 to 1950. In the past four years over 300 papers have appeared concerning technical modifications, applications and limitations of the method. This extensive literature has been comprehensively surveyed in recent articles by Wunderly,¹ McDonald et al.,^{2,3} Grassman and Hannig,⁴ and Kunkel.⁵ No attempt will be made here to review this material. Rather, it is our purpose to report the results obtained using filter paper electrophoresis for the study of the serum and urine proteins of thirty-five cases of multiple myeloma assembled in the course of the past year from the wards of the Francis Delafield and Presbyterian Hospitals.

Since all of these patients were studied by iliac bone marrow aspiration, skeletal x-rays, serum protein fractionation by salt and/or alcohol solubility technics, and urine tests for Bence-Jones proteins, it has been possible to appraise the diagnostic specificity and sensitivity of these procedures as compared with serum and urine electrophoretic studies. In addition, certain special studies of the myeloma serum and urine proteins which may provide an increased understanding of their chemical composition and their relationship to each other (i.e., serum to urine) will be described. Two additional cases (not included among the thirty-five documented

myeloma cases), which exhibited certain unusual clinicopathologic features linking them to the category of malignant lymphomas, will be dealt with in detail. A third case, which demonstrated the value of serial study of the serum electrophoretic pattern for appraisal of the efficacy of a therapeutic regimen, will also be described.

MATERIALS AND METHODS

As noted previously, a considerable portion of the extensive literature pertaining to zone electrophoresis deals with technical modifications of this basically simple procedure. Virtually all investigators working with paper electrophoresis have made minor or major alterations in the technic in attempts to control several of the variables inherent in the method. This laboratory has employed a new technic for supporting the filter paper sheets. Because this modification has resulted in considerably sharper resolution of the protein fractions and greater uniformity and reproducibility of these patterns, a description of the apparatus will be given in this report.

The major modification consists in substitution of two methacrylate frames for the glass plate or large chambers commonly employed as the means for supporting the sheets or strips of filter paper. The assembled apparatus is diagrammed in Figure 1. The construction details of the lucite frames are shown in Figure 2. Since the upper and lower frames are identical, only one is shown. These frames are easily constructed by bonding the 1 cm. square lucite framing strips to the lucite base plate with chloroform or a similar methacrylate solvent. The dimensions shown in the diagram are

* From the Medical Service, Francis Delafield Hospital, and the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, N. Y. This work has been supported in part by an institutional grant from the American Cancer Society.

those which have been employed in the routine laboratory, and up to ten 0.005 ml. serum or urine samples can be accommodated. Considerably larger units have been found equally satisfactory provided that excessive sagging of the buffer-moistened filter paper is avoided.

(0.005 ml.) are applied to previously pencil-marked sites in a line down the middle of the paper. The upper plate is then set in place, thereby forming a chamber in which the paper is held suspended. A 25 pound weight (lead brick) presses the frames together and restricts the

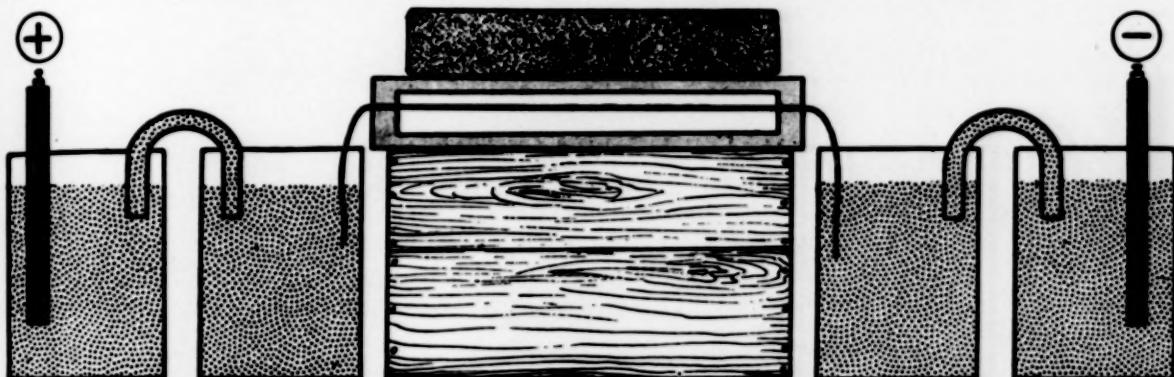


FIG. 1. Schematic diagram of the assembled electrophoresis apparatus with the filter paper (seen in cross section) held in suspension by the plastic frames.



FIG. 2. Construction details of the plastic frame. Since the two plates are identical, only one is shown. The filter paper sheet is cut slightly narrower than the frame.

As indicated in Figure 2, the filter paper (Whatman 3 MM) is cut slightly narrower than the plates, and sufficiently long to project into the buffer-filled vessels. The filter paper is moistened with buffer solution, blotted to dampness and suspended over the bottom plate. Sagging is avoided by pre-applying high vacuum-type silicone stop-cock grease to the rims of the plates parallel to the direction of migration, and firmly pressing the edges of the paper against this heavy grease. No grease is applied to the end rims. Aliquots of the serum or urine

extent of wetting of the paper. Evaporation from the sides is prevented by sealing with additional stop-cock grease. Prior to applying the voltage, a thirty-minute period is allowed for the moisture content of the chamber to come to equilibrium. Vapor saturation of the chamber with moisture-bead formation on the inside surfaces of the plates is usually apparent within this thirty-minute period. Veronal buffer, pH 8.6, $\mu = 0.1$, is routinely employed. A constant potential of 5 volts/cm., applied for a six-hour period, results in an albumin migration of approxi-

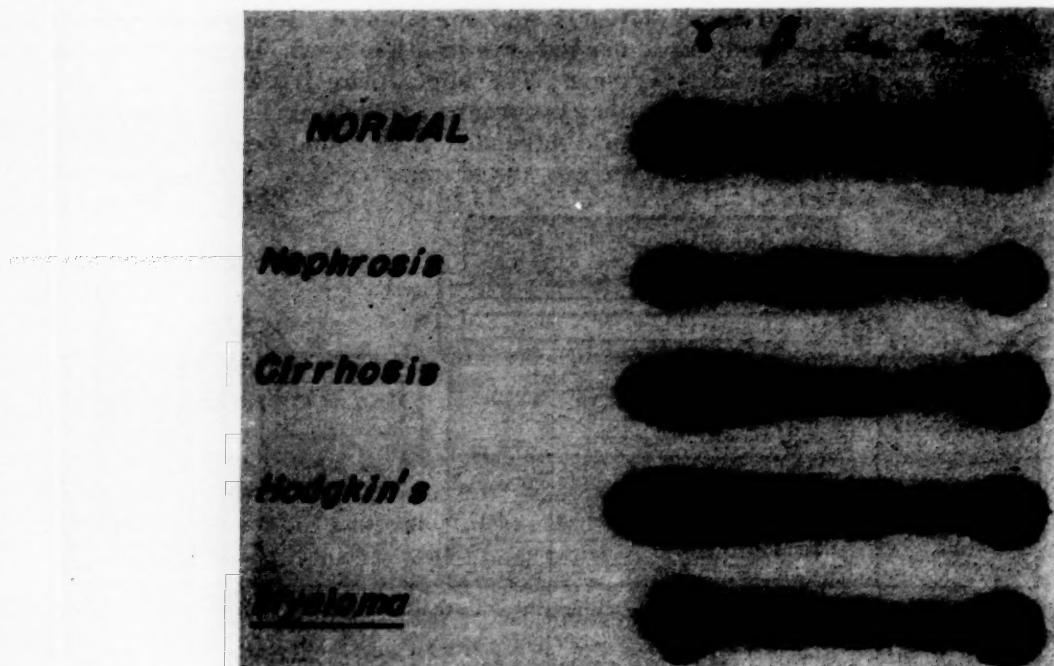


FIG. 3. Serum electrophoretic patterns in four disease states associated with marked protein abnormalities. The homogeneity of the myeloma globulin is readily distinguishable from the diffusely increased gamma globulins of the serum in cirrhosis and Hodgkin's disease.

mately 6 cm. and only minimal electro-osmotic cathodal displacement.

At the completion of the six-hour run the protruding ends of the filter paper are torn off, prior to separation of the plates and removal of the paper. To avoid distortion of the patterns in the course of evaporation and drying, the paper is either stained immediately while still moist, or is dried rapidly by exposure to 100°F. for ten minutes prior to staining. A 1 per cent solution of bromphenol blue in a saturated alcoholic solution of mercuric chloride is employed for routine staining.

Urine specimens were treated according to the procedure of Slater and Kunkel⁶ and were concentrated by dialysis in a cellophane membrane, against a concentrated (25 per cent) solution of polyvinylpyrrolidone.* The degree of dialysis concentration varied in accordance with the initial protein content of the specimen. Since the qualitative nature of the proteinuria in myeloma is of greater diagnostic significance than the absolute amount of protein excreted, quantitation of the proteinuria is unnecessary.

Several methods have been described for the quantitation of protein fractions separated by

* Kindly supplied by Schenley Laboratories, Inc., New York, N. Y.

filter paper electrophoresis. These technics have varying limits of accuracy and reproducibility. They include various procedures of dye-elution, absorption photometry, planimetry, etc. Although these procedures have usefulness in many laboratory applications of the technic, routine clinical requirements do not, in the opinion of the authors, warrant the additional time and effort involved. In all our routine reports a 0.005 ml. aliquot of fresh, pooled normal serum is run adjacent to the patient's sample, and the submitted report includes both patterns for direct comparison.

RESULTS

The characteristic electrophoretic homogeneity of so-called "myeloma proteins" is seen in Figure 3, and is readily distinguishable from the other pathologic patterns which exhibit diffuse globulin abnormalities. It is this same characteristic homogeneity which distinguishes myeloma urine proteins from other pathologic proteinurias. This is shown in Figure 4 in which a myeloma patient's serum and urine patterns are compared with the serum and urine patterns of a patient in the nephrotic phase of chronic glomerulonephritis. The urine of the nephrotic patient obviously contains significant amounts of

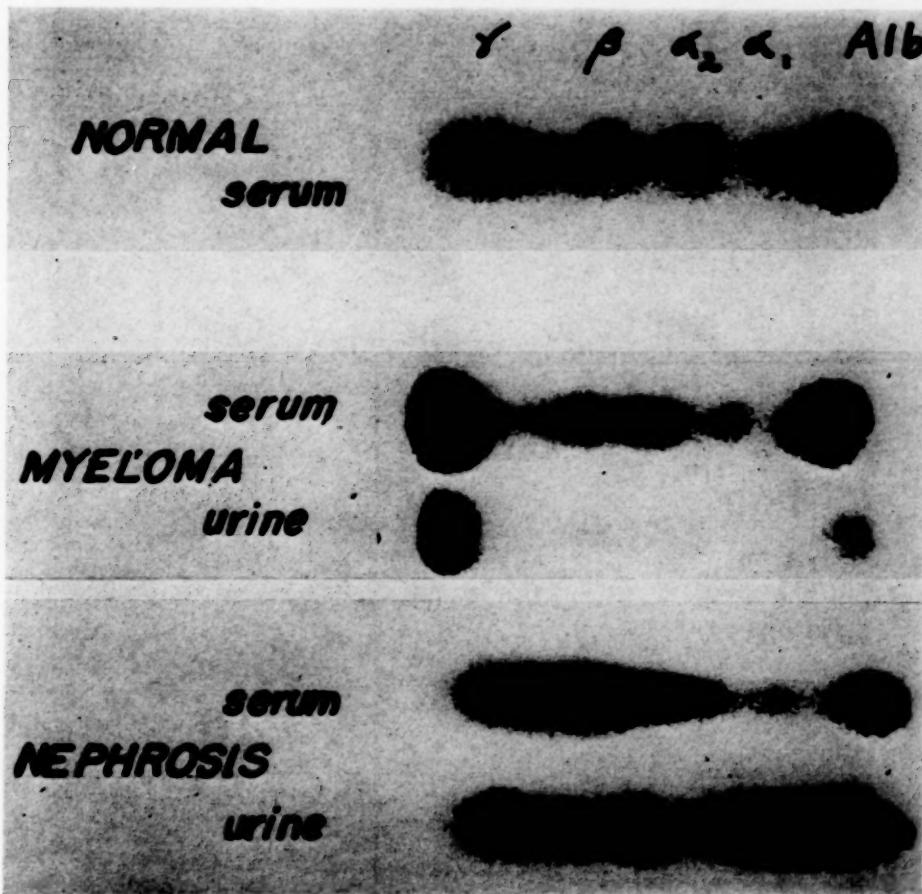


FIG. 4. Comparison of the serum and urine protein patterns of myeloma and nephrosis. The nephrotic urine is seen to contain all the protein constituents of the serum whereas the myeloma urine shows a large amount of homogeneous globulin and a small quantity of albumin.

all of the serum protein constituents whereas the characteristic myeloma urine shows a discrete and dominant globulin component. Presumably depending upon the degree of renal damage, the urine in myeloma may show varying amounts of other serum constituents in addition to this homogeneous globulin and, as will be seen later, some cases with diagnostic serum patterns show non-specific urine protein patterns.

As depicted in Figure 5, the electrophoretic mobility of these myeloma serum and urine proteins may range from that of a "slow γ " globulin to a more "rapid β " component. In our experience there have been no discernible clinical or pathologic characteristics associated with any particular globulin mobility. The relative mobilities of the serum and urine proteins in individual cases will be considered subsequently.

The results of these serum and urine electrophoretic studies in the thirty-five myeloma cases

have been grouped into four categories, examples of which are shown in Figure 6. These categories are as follows: Group I (seventeen cases) both serum and urine "positive," (i.e., showing an electrophoretically homogeneous globulin abnormality); Group II (eleven cases) serum "positive," urine negative. Included in this group are five patients whose urine contained appreciable amounts of albumin and "non-myeloma" globulin. Group III (seven cases) urine "positive," serum non-diagnostic; Group IV, no characteristic abnormality in either the serum or urine. To date the only two possible examples of the Group IV category have both been cases of localized plasmacytoma of a vertebral body, diagnosed by biopsy. Both cases showed no other osseous lesions by x-ray and marrow aspirations from other sites have failed to demonstrate myeloma cells. Neither of these cases has come to autopsy and thus the true extent of their disease is still undetermined. In all

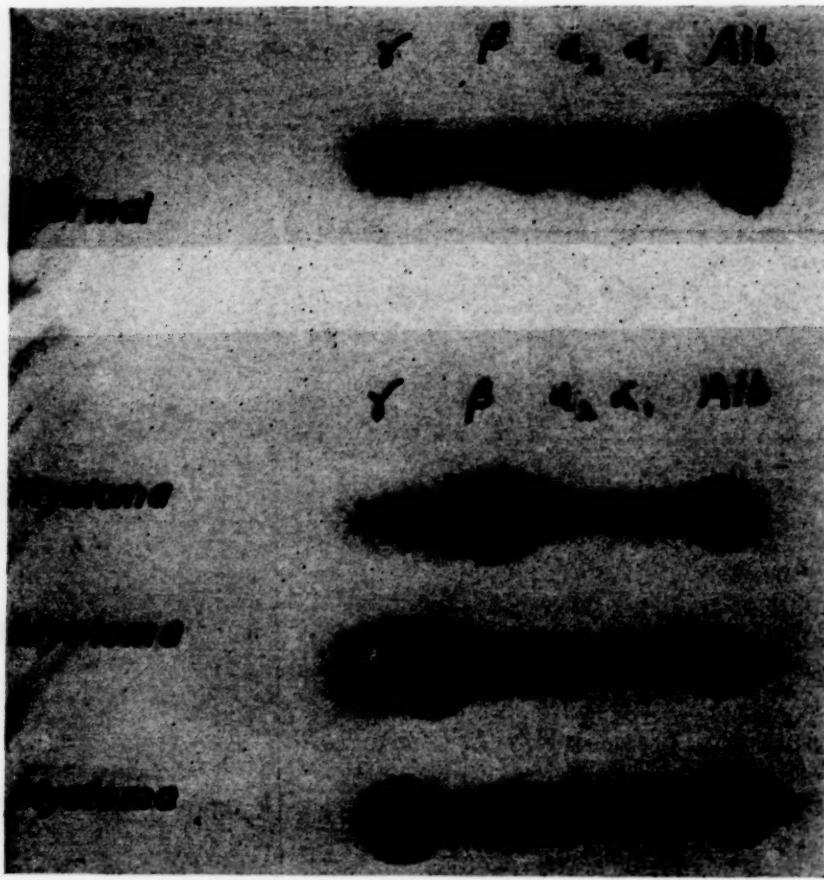


FIG. 5. Serum electrophoretic patterns from three myeloma patients, demonstrating the difference in mobility of the abnormal globulins in each case: the first corresponds to a "fast β "; the second, a "fast γ "; and the third, a "slow γ ."

other cases in which disseminated myeloma has been documented, a diagnostic protein abnormality has been found by electrophoresis. These results are summarized in Table I.

The electrophoretic mobility of the myeloma serum and urine proteins in each individual case is schematically depicted in Figure 7. As prior studies of large groups of cases have shown,⁷⁻¹⁹ the majority of the abnormal serum peaks occur in the gamma-globulin area (twenty-three of twenty-six serums), with fewer showing mobilities of beta globulin, or intermediate between beta and gamma. In this series no instance of a characteristic serum myeloma peak with alpha-globulin mobility, was encountered, although moderate increases in the alpha globulins of a non-specific nature have frequently been observed.

Figure 7 also shows the relative electrophoretic mobilities of the serum and urine constituents in those seventeen Group I cases which

TABLE I
SUMMARY OF SERUM AND URINE ELECTROPHORETIC STUDIES
AND URINE BENCE-JONES TESTING IN THIRTY-FIVE CASES
OF MYELOMA

Group	Characteristic Electrophoretic Abnormality	No. of Cases	No. of Cases with Bence-Jones Positive Proteinuria
I	Serum and urine	17 (49%)	12
II	Serum only	11 (31%)	0
III	Urine only	7 (20%)	4
		Total 35 (100%)	16 (46%)
IV	Neither serum nor urine	Possibly 2 cases of localized plasmacytoma	0

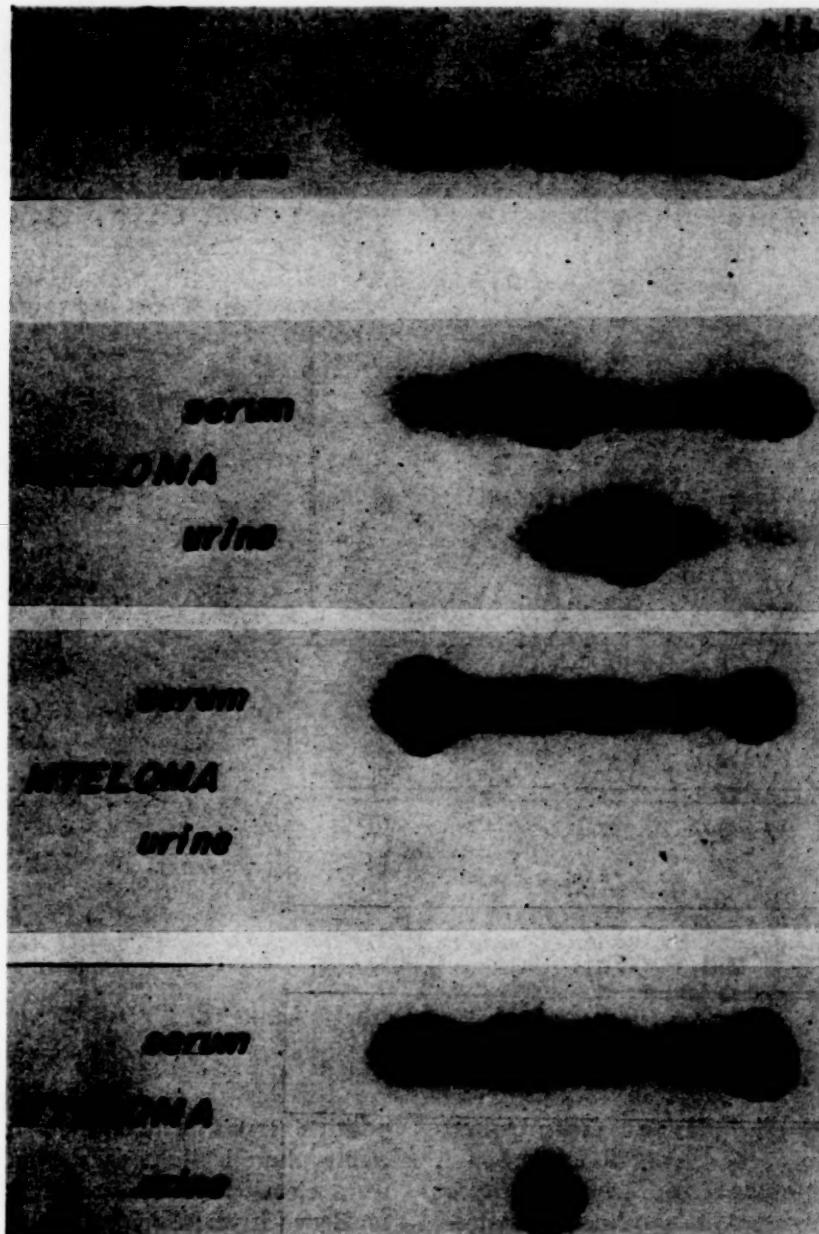


FIG. 6. Examples of the types of diagnostic abnormal protein patterns in myeloma. Group I, positive in serum and urine; Group II, serum only; Group III, urine only; Group IV, no characteristic abnormality. The serum pattern of the Group III example shows a suggestive but not diagnostic increase in the β globulin region.

exhibited abnormal proteins both in serum and urine. As has been observed in previous studies, the mobility of the urine protein is usually more rapid than that of the corresponding serum constituent (eleven of seventeen cases). Exceptions to this general rule, however, are not uncommon; the proteins in four of the seventeen Group I cases showed corresponding serum and urine mobilities, and in two instances the urine

protein was of slower mobility than the serum peak.

As already mentioned, several of the urine electrophoretic patterns in the patients of Groups I and III showed the presence of other serum protein constituents in addition to the discrete, diagnostically homogeneous "myeloma protein." Usually this additional proteinuria consisted of relatively small amounts of albumin, as seen in

the myeloma pattern in Figure 4. Even in the presence of larger quantities of the other serum proteins, however, the abnormal myeloma component has always been easily distinguishable.

All urine specimens in which appreciable amounts of protein could be demonstrated by

the serum electrophoresis and the urine Bence-Jones protein test. As expected, the urines of the five Group II cases which did not contain an electrophoretically characteristic myeloma globulin were all Bence-Jones protein negative. Thus, in summary, all urine samples giving

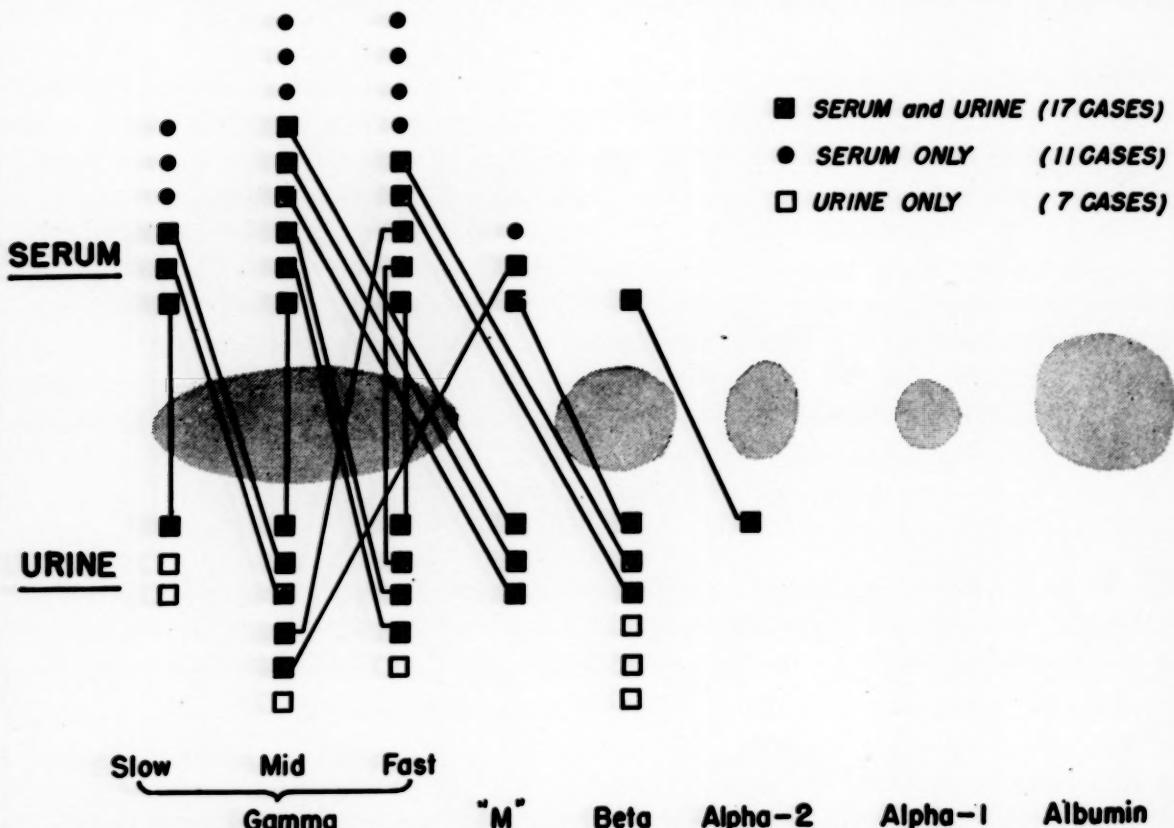


FIG. 7. Distribution diagram of the electrophoretic mobilities of the abnormal serum and urine proteins in thirty-five cases of multiple myeloma.

sulfosalicylic acid precipitation were subjected to the usual tests for Bence-Jones protein. The results, as summarized in Table I, were as follows: of the twenty-four urine specimens (seventeen in Group I; seven in Group III) which were "electrophoretically positive," a classic Bence-Jones protein reaction was demonstrable in sixteen (66 per cent). The remainder (eight specimens) showed no precipitation below 60°c. after acidification to pH 5.5, although protein was demonstrable by 20 per cent sulfosalicylic acid precipitation. Particularly significant were three of the Group III cases which were Bence-Jones protein negative but electrophoretically positive. Because of the normal serum protein patterns in these three cases the diagnostic protein abnormality would most certainly have been missed had the work-up included only

positive tests for Bence-Jones protein were electrophoretically positive, whereas eight urines were electrophoretically positive and negative for Bence-Jones protein.

CASE REPORTS

As stated previously, the diagnosis of multiple myeloma is considered to have been well documented in all of the thirty-five cases included in this series. Two additional patients have been observed whose serums contained an abnormal homogeneous protein but whose clinical and pathologic features were not characteristic of myelomatosis. These cases have not been included in the group of thirty-five documented cases of myeloma but will be described in detail because they may provide a slightly better insight into the relationship of

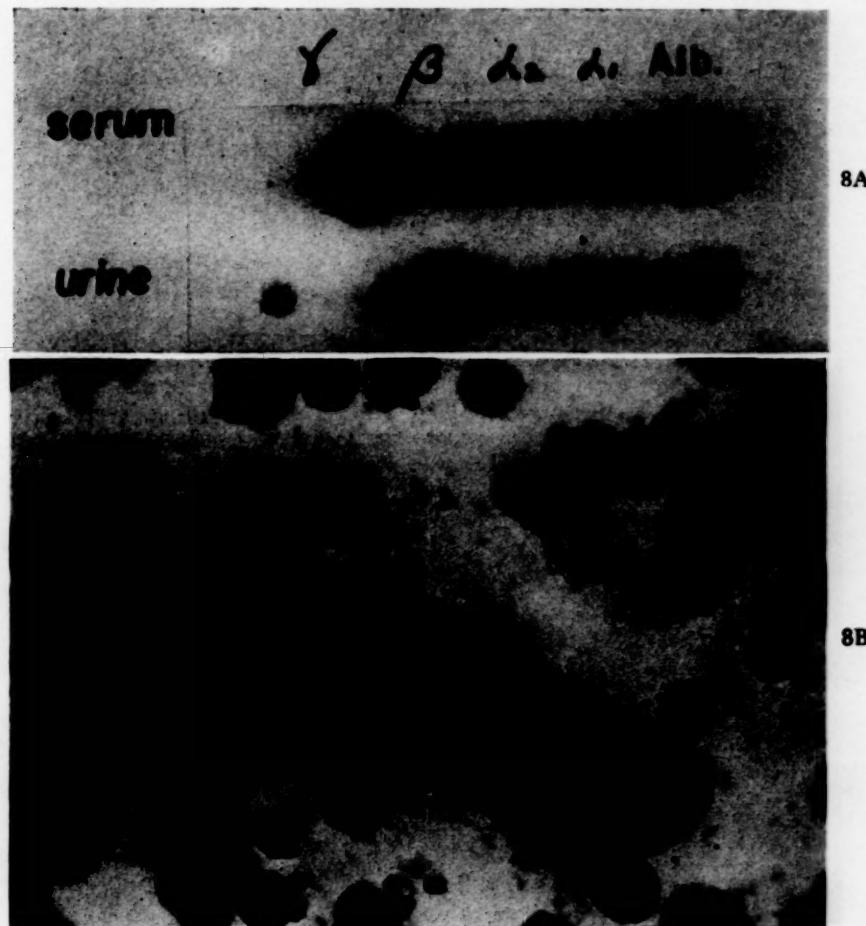


FIG. 8. Case 1. B. M. (FDH No. 3447). A, serum and urine electrophoretic patterns; B, photomicrograph of bone marrow cytology.

myeloma to lymphosarcoma and chronic lymphatic leukemia. A third case will also be cited in some detail because it demonstrates the value of serial study of the serum electrophoretic pattern for appraisal of the efficacy of a particular experimental therapeutic regimen.

CASE 1. B. M. (FDH No. 3447), a fifty year old white man with an essentially non-contributory past history, was initially admitted to Presbyterian Hospital with a six-week history of weakness and increasingly severe low back and right lateral chest pain markedly intensified by movement. Physical examination was negative except for pallor and tenderness of several ribs and vertebral spines. There was no lymphadenopathy or hepatosplenomegaly.

Laboratory data revealed the following: Hemoglobin 6.0 gm.; red blood count 2.88 million; white blood count 6,900 with polymorphonuclears 52; lymphocytes 42; monocytes 4; eosinophils 2; no abnormal lymphocytes or

plasma cells. Erythrocyte sedimentation rate 135 mm./hour. Urinalysis: protein 1+; Bence-Jones negative; non-protein nitrogen 37 mg. per cent; uric acid 9.4 mg. per cent. Serum proteins: A/G 4.3/3.8 (Majoor modification of the Howe technic); 3.5/4.2 (Pillemer and Hutchinson). The serum and urine electrophoretic patterns are shown in Figure 8, demonstrating characteristic abnormalities in both. Skeletal x-rays disclosed disseminated osteolytic mottling of all visualized bones, with compression fracture of the body of L₁. Two iliac crest marrow aspirations and one sternal marrow biopsy all disclosed extensive replacement of normal structure with neoplastic cells having the appearance of young lymphoblasts. None of these cells resembled abnormal plasma cells. A characteristic marrow area is also shown in Figure 8.

In summary this case exhibited all of the characteristic clinical and biochemical features of multiple myeloma although the histologic

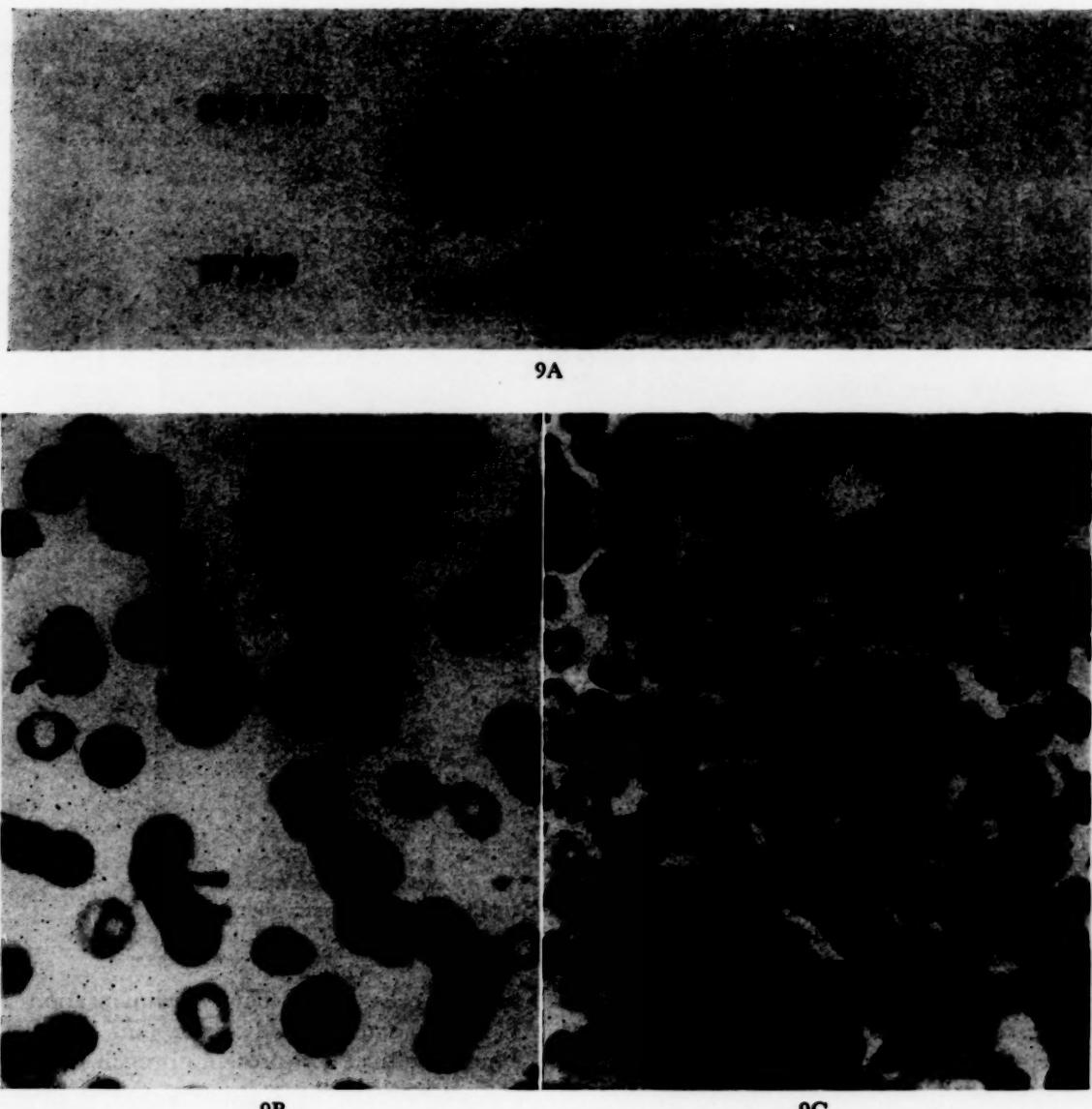


FIG. 9. Case II. S. K. (FDH No. 4421). A, serum and urine electrophoretic patterns; B, peripheral blood smear; C, cervical lymph node cytology.

appearance was that of lymphosarcoma. To be sure the possibility exists that some unexamined areas of involvement may demonstrate the typical cytology of plasma cell proliferation.

CASE II. S. K. (FDH No. 4421), a sixty-two year old white housewife, was admitted to Delafield Hospital with an eighteen-month history of weakness and painless cervical and axillary adenopathy. One year previously a blood count had disclosed hemoglobin 8.6; white blood count 10,450 with neutrophils 39, lymphocytes 61. A dorsal spinous process marrow aspiration (L_1) at that time had shown a marked increase in lymphocytes. No abnormal lympho-

cytes were noted. Biopsy of a cervical node similarly revealed extensive proliferation of small lymphocytes, and a diagnosis of chronic lymphatic leukemia was made. For the ensuing year therapy consisted only of transfusions, and during this period the total white count never rose above 20,000.

When first seen at Delafield Hospital examination disclosed generalized lymphadenopathy, with nodes up to 7 cm. in diameter in the axillary and supraclavicular regions. The spleen was enlarged to 16 cm. below the costal margin; the liver was palpable to 10 cm.

Laboratory data revealed the following:

Hemoglobin 6.0 gm.; red blood count 2.81 million; white blood count 10,600; neutrophils 25; monocytes 4; lymphocytes 71, with approximately half of these lymphocytes appearing immature, and somewhat resembling plasma cells. (Fig. 9.) Erythrocyte sedimentation rate 156 mm./hour. Urinalysis: Protein 1+; Bence-Jones protein negative. Serum proteins: A/G 2.9/4.8 (cold methanol method of Pillemer and Hutchinson). The serum and urine electrophoretic patterns (Fig. 9) revealed a markedly homogeneous globulin abnormality in both. Biopsy of a cervical lymph node (Fig. 9) disclosed marked proliferation of immature lymphocytes, with no apparent increase in plasma cells. Skeletal x-rays were negative except for a partial collapse of the body of L-1, strongly suggestive of tumor involvement.

In contrast to the previous case, in which the clinical features and distribution of lesions were typical of myelomatosis, this second patient's clinical pattern, i.e., the generalized lymphadenopathy, hepatosplenomegaly and lymphocytosis, is most compatible with the diagnosis of chronic lymphatic leukemia. Although several previous studies of the serum electrophoretic patterns in lymphatic leukemia²⁰⁻²² and the malignant lymphomas^{21,23,24} have failed to reveal any consistent characteristic protein abnormalities comparable to those of myeloma, occasional cases of both of these disease entities have been observed to display abnormal serum globulins (including cryoglobulins) akin to those found in myeloma.^{14,22,25-28} The generally accepted thesis of a primordial cellular relationship between the plasma cell and the lymphocyte makes it not unreasonable to postulate that "intermediate" or transitional cases, such as the two here presented, would occur.

Since the weight of experimental evidence favors the thesis that the abnormal myeloma proteins are truly products of the neoplastic cells, electrophoretic studies are not only of diagnostic value but also of use in following the progression of the disease and evaluating any therapeutic regimen. The third case demonstrates this application particularly well in that the patient has been under constant observation in the hospital for over one year. As noted in a prior report,²⁹ this patient's abnormal serum protein was a cryoglobulin, making the serial protein studies particularly instructive.

CASE III. K. S. (FDH No. 3000), a fifty-eight year old white man, was admitted to

Delafield Hospital fifteen months previous to this report and has been hospitalized and under continual study throughout this entire period. He presented with a two-year history of increasingly severe low back pain and left hip pain. Prior work-up at another hospital had disclosed anemia, 1+ proteinuria, hyperproteinemia and widespread osteolytic lesions, with pathologic fractures of the left femoral neck and the second lumbar vertebra. Because two iliac bone marrow aspirations had failed to provide adequate histologic documentation, an osteolytic rib lesion was biopsied and the diagnosis of plasma cell myeloma was thus established.

Physical examination initially showed the patient to be markedly emaciated, dehydrated and disoriented. The left leg showed an internal rotation deformity with abnormal prominence of the left femoral trochanter. Several other bony sites were tender to pressure. There was no lymphadenopathy or hepatosplenomegaly.

Laboratory data revealed the following: Hemoglobin 7.5 gm.; red blood count 2.82 million; white blood count 9,050; polymorphonuclears 68; lymphocytes 23; eosinophils 1; platelets 121,000. Erythrocyte sedimentation rate 145 mm./hour. Urinalysis: Protein 1+, Bence-Jones negative; serum non-protein nitrogen 32 mg. per cent; uric acid 5.8 mg. per cent; calcium 11.8 mg. per cent; phosphorus 2.8 mg. per cent. Alkaline phosphatase 2.0 Bodansky units per cent.

Shortly after admission it was noted that the patient's serum exhibited the remarkable property of solidifying when exposed to refrigerator temperature, i.e., approximately 10°C. for ten minutes. This cold-gelation was shown to be due to the presence of a large quantity of abnormal globulin, so-called cryoglobulin. At this time the total serum protein content, as calculated from the standard Kjeldahl nitrogen method, was 11.0 gm. per cent; A/G 1.6/8.4 gm. per cent (cold methanol method of Pillemer and Hutchinson). When the total protein was measured by the biuret reaction a value of 8.0 gm. per cent was obtained, i.e., 3.0 gm. lower than the Kjeldahl value. This 3.0 gm. discrepancy was shown to be entirely due to the globulin fraction, indicating that the cryoglobulin was unique in its chemical composition with respect to its nitrogen content relative to the number of peptide linkages available for biuret reaction. By both boundary and filter paper electrophoresis, the cryoprotein was

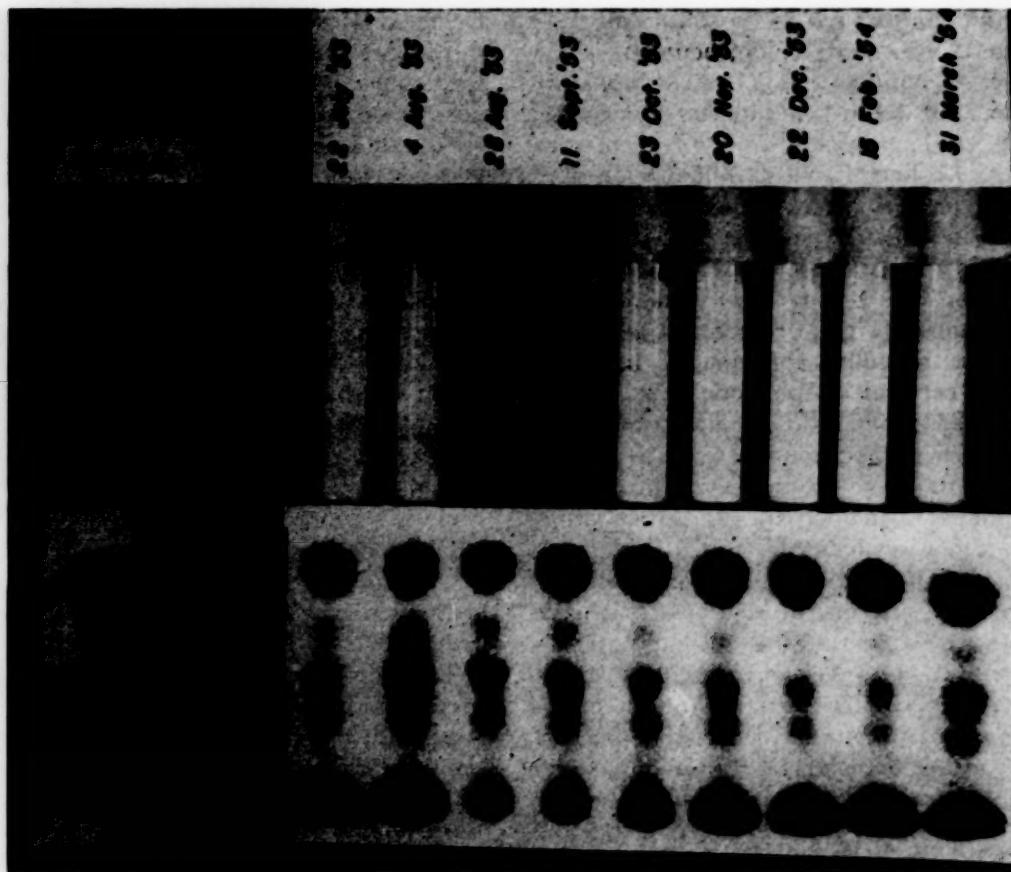


FIG. 10. Case III. K. S. (FDH No. 3000). Serial samples of serum photographed at 10°C. to demonstrate the cryoglobulinemia. The filter paper electrophoretic patterns corresponding to each of the serum samples are also shown. The striking decrease in the abnormal gamma globulin with temporary disappearance of the cryoprecipitation is apparent in the August 28th and September 11th samples. A tube of normal serum, and a normal serum electrophoretic pattern are shown for comparison.

identified as an homogeneous gamma globulin ($\mu = 2.2 \times 10^{-5}$ cm.²/volt. sec.) The sedimentation constant (S_{20}) by ultracentrifugation was 5.7.

In the fifteen-month period of hospitalization the patient has received two therapeutic trials of 6-mercaptopurine (6-MP) followed by a seven-month course of urethane which is still in progress. Paper electrophoretic patterns of the serum were made at frequent intervals, and the serum samples stored frozen for subsequent serial comparisons. Figure 10 shows nine serum samples photographed at 10°C. to demonstrate the cold-gelation along with the corresponding electrophoretic patterns.

Coincident with the first course of 6-MP (August, 1953), there was a striking improvement in the serum protein abnormalities, with a marked decrease in the cryoglobulinemia. After two weeks of 6-MP the total serum protein had

decreased to 6.0 gm. per cent, with correspondence between the Kjeldahl and biuret values. Paper electrophoresis (August 28, 1953, sample of Figure 10) confirmed the decrement in the abnormal γ constituent, and the cryoprecipitation was no longer demonstrable.

The development of leukopenia, with depression of the white blood count to 2,800, forced discontinuation of 6-MP after four weeks. Although the white blood cell count promptly returned to normal limits (within two weeks), the cryoglobulin also returned in progressively increasing concentration.

A second trial of 6-MP was instituted in November, 1953, and was ultimately carried to a dosage level two and one-half times greater than the initial course, with virtually no effect on either the peripheral blood count or the serum proteins. This agent was discontinued after six weeks.

After another six-week period without specific therapy a course of urethane was started in February, 1954. This has been continued up to the present time (September, 1954) at a dosage level of 3.6 gm. O.D. Thus far there has been only a minimal decrease in the cryoglobulinemia, and cold gelation is still readily demonstrable. Although he remains bedridden, there appears to have been a distinct slowing of the progression of his osseous lesions and he is entirely free of pain. In the past five months, while receiving urethane, the peripheral blood count has been maintained without transfusions, in the range of 11.5 gm. of hemoglobin, 5,500 white blood cells and 200,000 platelets.

This case is considered to be of particular interest in that it demonstrates the usefulness of the paper electrophoresis technic for the serial study of serum abnormalities as another method for objective evaluation of drug therapy in myeloma. Although the temporal relationship between the initial improvement in serum proteins and the administration of 6-mercaptopurine strongly suggests a causal relationship, failure to obtain any comparable results in five additional cases of myeloma treated with this agent precludes any general conclusions.

COMMENTS

With regard to analysis of the serum proteins in myeloma it has been well established that electrophoresis, either by the classic Tiselius technic or with the simpler filter paper methods, represents the best of the available protein fractionation procedures for diagnostic purposes.⁷⁻¹⁹ A survey of the larger published series of myeloma cases (Table II) discloses that approximately 75 per cent of myeloma patients may be expected to show characteristic serum electrophoretic patterns. An additional 18 per cent of cases, failing to show a characteristic serum abnormality, may be anticipated to have demonstrable Bence-Jones proteinuria, thereby extending the laboratory's diagnostic score to slightly over 90 per cent. The results obtained in this present series of thirty-five cases, in which electrophoresis of the urine proteins was routinely performed, would indicate that a still higher score may be possible by demonstrating diagnostic urine electrophoretic patterns in samples which fail to give a positive Bence-Jones protein reaction. Several investigators^{9-11,12,14,16,20} have stressed the importance of careful attention to conditions of pH, concentration and tempera-

ture for demonstration of the classic thermolability reaction, and have noted the frequency with which excessive amounts of albumin and other protein constituents may mask lesser Bence-Jones proteinuria. Our experience would indicate that filter paper electrophoresis of urine

TABLE II
SURVEY OF THE LARGER SERIES OF REPORTED MYELOMA CASES*

Reference	Total Cases	Cases with Diagnostic Serum Electrophoretic Patterns	Cases with Normal Serum and Bence-Jones Proteinuria	Diagnostic Score of Serum E-P plus Urine Bence-Jones (%)
Adams et al. ¹¹	30†	21	7	93
Putnam and Udin ¹²	25	21	4	100
Rundles et al. ¹³	30	25	3	93
Snapper ^{16‡}	44†	31	10	93
Reineg and Stern ^{17‡}	53	35	13	90
Griffiths and Brews ¹⁸	20	19	1	100
Conn and Klaskin ¹⁹	18	14	2	88
Total.....	220	166 (75%)	40 (18%)	93

* Serum electrophoresis (either Tiselius or filter paper method) and results of urine Bence-Jones testing were reported in these cases.

† A greater total number of cases were reported in these series but the serum electrophoretic patterns and urine Bence-Jones results were presented in only the number of cases noted.

‡ Since several of the cases in these two series were patients at the Mount Sinai Hospital, there was some reduplication.

proteins possibly is less subject to technical error than the seemingly simple Bence-Jones test. It should be emphasized that no instance has been found in this present series, or in the reviewed literature, in which a Bence-Jones protein positive specimen has failed to show a diagnostically homogeneous protein peak by electrophoresis.

Whereas electrophoresis of urine samples is feasible with the boundary Tiselius system, and has been carried out by several groups investigating myeloma,^{9,12,14,20,21} the prolonged and cumbersome dialysis preparation of specimens required for adequate resolution in a free system virtually precludes the use of this technic for routine clinical purposes. The fact that the filter paper method obviates the need for this careful salt equilibration constitutes one of the major advantages of this modification.

Although quantitation of these abnormal serum and urine globulins (as well as the other serum constituents) would seem desirable, the experience of this laboratory has indicated that this is unnecessary for diagnostic purposes. It must be appreciated that many of the methods

available for quantitation of protein fractions may well be subject to considerable error when applied to abnormal constituents such as these myeloma globulins.

In their recent study of the serum electrophoretic patterns in eighteen cases of myeloma, Conn and Klatskin¹⁹ compared the quantitative results obtained from the dye-elution method of filter-paper analysis with the planimetric quantitation of the Tiselius electrophoretic patterns. Although there was generally good correlation between the quantitative results of the two methods, appreciable discrepancies were found in several samples. As stated,¹⁹ these discrepancies are not surprising when one considers that the two methods are based on two entirely different physicochemical properties, i.e., the refractive index and the dye-binding capacities.

Recent studies carried out in this laboratory³² strongly suggest that the abnormal myeloma serum globulins may be conjugated glycoproteins, i.e., there may be a significant amount of carbohydrate bound to the protein component. This could well account for an appreciable error in the application of any quantitative technic based upon non-conjugated proteins such as serum albumin and normal gamma globulin.

Experimental results such as those of Martin,³³ Lane³⁴ and Miller et al.,³⁵ in which abnormal globulins have been isolated from myeloma tissue with electrophoretic characteristics corresponding to the abnormal serum constituents of the patient from whom the tissue was obtained, lend strong support to the thesis that these proteins are truly products of the neoplastic cells. Despite their apparent common origin, however, marked differences have been documented in the physicochemical properties of these abnormal proteins in individual cases of myeloma. Although the abnormal serum globulin in two cases may exhibit the same electrophoretic mobility, they may be found to differ in molecular weight and solubility properties,^{7,8} 13–15,30 amino acid composition³⁶ and immunological characteristics.^{37–39} Similar studies of the urine proteins in myeloma^{40–42} have disclosed comparable differences in these proteins from case to case. It would thus appear that the abnormal myeloma proteins are almost never identical with respect to all of their physicochemical properties in any two cases of this disease.

Another unresolved question concerns the

nature of the relationship between the myeloma serum and urine proteins when both are present in a particular case. As yet, it is undetermined whether the serum and urine constituents are two distinct proteins, independently elaborated by the myeloma cells, or whether the protein excreted in the urine represents a smaller molecular fragment of the parent serum globulin.

As mentioned previously, current studies in this laboratory³² suggest that the myeloma serum globulins may be conjugated glycoproteins, containing a significant quantity of carbohydrate bound to the protein component. This has been postulated from the observation that these globulins, separated by filter paper electrophoresis, stain intensely with the periodic acid-Schiff technic.^{43,44} In contrast to this behavior of the serum constituents, the urine myeloma proteins, electrophoretically separated, fail to give a positive Schiff reaction, suggesting that they are devoid of any significant carbohydrate component. Obviously, these observations do not provide a definitive answer to the question of the inter-relationship between these myeloma serum and urine proteins. The suggested systematic difference in chemical composition might well be interpreted as further evidence in favor of independent elaboration of two distinct proteins. However, another possible mechanism must also be considered, i.e., that a carbohydrate constituent may be removed from the parent serum protein, leaving a smaller molecular fragment, filtrable through the glomeruli and excreted in the urine. Since this removal of an electro-neutral, uncharged portion of the serum protein would possibly leave the smaller fragment with a relatively greater net electrical charge, this mechanism might offer an explanation for the usual finding (Fig. 7) that the electrophoretic mobility of the urine protein is greater than that of the corresponding serum protein from the same case. These possibilities are currently under investigation.

SUMMARY

1. Filter paper electrophoretic analysis of the serum and urine proteins in thirty-five cases of multiple myeloma has been carried out. In all thirty-five cases characteristic protein abnormalities were observed; seventeen cases showing homogeneous peaks in both the serum and urine, eleven cases in serum only, seven cases in urine only.

2. All urine samples showing a positive Bence-

Jones protein reaction also displayed a characteristic electrophoretic abnormality. Eight of the twenty-four electrophoretically positive urine samples were Bence-Jones protein negative by the usual tests. Because of the relative simplicity of urine electrophoresis by the filter paper technic, this test is considered to be a practical clinical procedure.

3. Two cases are presented in detail because certain atypical clinicopathologic features of their disease patterns possibly assist in an understanding of the relationship of the plasma cell dyscrasias to chronic lymphatic leukemia and the lymphosarcomas. A third case, which demonstrates the value of serial electrophoretic studies in myeloma, is also presented.

4. Consideration is given to some of the physicochemical properties of the myeloma serum and urine proteins, and to the possible nature of their interrelationship.

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Review

Influence of Age and Vascular Disease on Cerebral Hemodynamics and Metabolism*

JOSEPH F. FAZEKAS, M.D., JACK KLEH, M.D. and FRANK A. FINNERTY, M.D.
Washington, D. C.

Of the 250 cc. of oxygen consumed per minute by the average-sized man, approximately 50 cc. are utilized by the brain to meet its metabolic demands. Since the brain is largely an aerobic organ, it depends completely upon its circulation for a constant supply of oxygen. It has been estimated that approximately 15 per cent of the total cardiac output is diverted to the brain.¹ The high metabolic activity of the cerebral cells and their extreme dependence upon the circulating oxygen makes them unusually susceptible to ischemia of even short duration, those cells with the highest metabolic requirements being most vulnerable.² In addition to oxygen the cerebral cells are dependent upon their circulation for other substances essential for their metabolic activity.

The oxygen consumed by the brain is for the most part utilized for the oxidation of glucose. Since the carbohydrate stores of the brain are inadequate to support its oxidative needs for any appreciable time,³ glucose must also be constantly extracted from the cerebral circulation. Various vitamins and minerals are recognized as essential components of various co-factors necessary for the oxidation of glucose.⁴ These and undoubtedly many other equally essential substances are made available to the cerebral cells via their circulation. In acute situations in which the cerebral circulation is markedly impaired (locally or generally) it is the lack of oxygen that is primarily responsible for

the cellular deterioration that rapidly ensues since its turnover rate is greater than that of the other substances extracted from the cerebral circulation.

Any organ so dependent on its circulation for its energy supplies must have compensatory mechanisms to protect it against fluctuations in the rate of its blood flow if it is to function efficiently. If the rate of cerebral blood flow is reduced, the extraction of glucose, oxygen and other substances from the arterial blood traversing the brain may be greatly increased. Under normal conditions the cerebral arteriovenous oxygen difference is approximately 7 volumes per cent (arterial oxygen content, 19 volumes per cent; venous oxygen content, 12 volumes per cent). With a marked reduction in the rate of cerebral blood flow the oxygen content of the cerebral venous blood may fall to as low as 2 volumes per cent, indicating extraction of almost all the available circulating oxygen. If the rate of cerebral blood flow is reduced so that cerebral oxygen delivery falls below the minimum requirements, acid intermediates from anaerobic processes accumulate and dilate the cerebral vessels. If these compensatory mechanisms become inadequate and the cerebral vascular delivery is not corrected, functional disturbances and/or structural damage to the cerebral cells rapidly result.⁵

The delivery and utilization of substances regularly required by cerebral cells for energy

* From the Georgetown and George Washington University Medical Divisions, District of Columbia General Hospital, Washington, D. C. This investigation was supported by a research grant (PHS B-178) from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service, and by a research grant from the United Cerebral Palsy Foundation. Parts of this paper were presented at a conference on Cerebral Vascular Disease held under the sponsorship of the American Heart Association in Princeton, N. J., from January 24 to 26, 1954.

production may be impaired at several levels: (1) vascular, (2) intercellular and (3) cellular.

1. *Vascular.* The substances may not gain access to the circulation or, because of general or local cerebral circulatory disturbances, they may not reach the brain capillaries.

2. *Intercellular.* The movement of solutes from the plasma to the cerebral intercellular fluid is undoubtedly influenced by the so-called "blood brain barrier." The importance of maintaining a highly stable intercellular environment for cerebral cells need not be emphasized. Tschirgi⁶ looks upon the blood brain barrier "as a second order homeostatic mechanism for maintaining a central neuronal milieu more constant than is possible by blood alone." There is still considerable discussion as to whether this selective permeability is governed by the perivascular glial membrane of Held or by the endothelium of the cerebral vessels. It is conceivable that as a result of cerebral vascular disease this specialized regulatory mechanism may be so disrupted that the diffusion of essential substances from the plasma to neuronal surfaces may be impaired because of altered osmotic gradients. It is also possible that as a result of the breakdown of the blood brain barrier substances foreign to cerebral cells may gain entrance and exert an inhibitory effect on their metabolic activity.

3. *Cellular.* Finally, the basic disturbance may be at the cellular level and even though the necessary substrates are delivered in adequate concentration, energy production or utilization may be impaired. Diminished energy production may result from interference with substrate oxidation because of enzyme or coenzyme deficiencies. If the disturbance is of sufficient magnitude, there may be a diminished oxygen utilization, reduced CO_2 production and an accumulation of certain intermediates depending on the locus of the metabolic block. A reduction of the rate of cerebral blood flow in such instances may be secondary to the decreased metabolic demands of the cerebral cells.

Cerebral arteriosclerosis is the most common cause of cerebral vascular insufficiency. In order to evaluate the effects of vascular disturbances on cerebral hemodynamics and metabolism it is important to determine separately if possible (1) the effects of aging, (2) the influence of hypertension and (3) the effects of focal and/or generalized cerebral vascular insufficiency on subjects of various age groups.

Since considerable work has been done on these problems we are presenting our data along with those of other investigators. It is hoped that definite conclusions may be reached and that the direction for future research indicated.

MATERIALS AND METHOD

The subjects of the present study were classified according to their clinical diagnosis and age: Group I, normotensive alert subjects under fifty years of age; Group II, subjects under fifty years of age with essential hypertension and no evidence of cerebral vascular insufficiency; Group III, subjects under fifty years of age with essential hypertension and cerebral thrombosis; Group IV, normotensive alert subjects over fifty years of age with no clinical evidence of cerebral vascular insufficiency; Group V, subjects over fifty years of age with hypertension and no clinical evidence of cerebral vascular insufficiency; Group VI, normotensive subjects over fifty years of age with cerebral thrombosis; Group VII, subjects over fifty years of age with hypertension and cerebral thrombosis; Group VIII, elderly normotensive subjects with psychosis; and Group IX, elderly hypertensive subjects with psychosis.

Scheinberg's and Stead's modification⁷ of Kety and Schmidt's procedure⁸ for the determination of cerebral blood flow (CBF) was applied in this study. The gas mixtures utilized were those described by the latter authors. Blood oxygen was determined by the manometric technic of Van Slyke and Neill.⁹ Mean arterial blood pressure (MAP) was obtained directly from the femoral artery by means of a damped aneroid manometer. The cerebral oxygen consumption (CMRO_2) and cerebral vascular resistance (CVR) were calculated as follows:

$$\text{CMRO}_2 (\text{cc. O}_2/\text{min.}/100 \text{ gm. of brain})$$

$$= \frac{\text{CBF} \times (\text{A-V})\text{O}_2}{100};$$

$$\text{CVR} (\text{mm. Hg/cc. blood}/\text{min.}/100 \text{ gm. of brain})$$

$$= \frac{\text{MAP}}{\text{CBF}}.$$

RESULTS

Table I presents the mean values obtained in the various groups of subjects studied. On line 1 the findings obtained in twelve young normotensive subjects are presented. The values obtained for cerebral blood flow, oxygen utilization and vascular resistance are in agreement with

those reported by other investigators^{8,10} for normal subjects of this age range.

In the second line the mean values for eight alert subjects with essential hypertension and no clinical evidence of cerebral vascular insufficiency are given. As indicated in the table, the

flow and cerebral metabolic rate are both significantly less ($P < .01$) when compared to values for hypertensive subjects of a similar age range without cerebral thrombosis (line 2). Despite the somewhat lower mean arterial pressure in this group of subjects, the cerebral

TABLE I
INFLUENCE OF AGE AND VASCULAR DISEASE ON CEREBRAL HEMODYNAMICS AND METABOLISM*

	Condition	No. of Subjects	No. of Observations	Age Range (yr.)	Mean Age (yr.)	MAP	CBF	CVR	CMRO ₂	Duration of Hypertension	Mental Status
1	Young normals	12	12	18-47	32	94	57.5	1.7	3.2	Alert
2	Young hypertensives	8	8	28-49	42	152	57.0	2.7	3.4	7 mo. to 12 yr.	Alert
3	Young hypertensives with cerebral thrombosis	15	15	34-49	44	138	41.6	3.4	2.5	6 mo. to 13 yr.	Alert
4	Elderly normotensives	13	18	57-99	80	94	47.7	2.1	2.7	Alert
5	Elderly hypertensives	17	17	52-84	68	150	48.7	3.2	2.8	2 to 20 yr.	Alert
6	Elderly normotensives with cerebral thrombosis	13	19	53-88	73	97	38.1	2.7	2.5	10 alert, 3 confused
7	Elderly hypertensives with cerebral thrombosis	51	58	50-101	65	137	40.5	3.6	2.3	3 mo. to 33 yr.	41 alert, 10 confused
8	Elderly normotensives with psychoses	13	16	72-92	83	94	38.4	2.6	2.2	Psychotic
9	Elderly hypertensives with psychoses	14	14	55-98	75	148	41.6	3.8	2.4	1 to 9 yr.	Psychotic

* In this and other tables, MAP signifies mean arterial blood pressure in mm. of Hg; CBF, cerebral blood flow in cc./min./100 gm. of brain; CVR, cerebral vascular resistance in mm. Hg/cc. blood/min./100 gm. of brain; CMRO₂, cerebral oxygen consumption in cc./min./100 gm. of brain.

known duration of the hypertension varied from seven months to twelve years. All subjects had retinal changes but none had papilledema. The only significant difference between the values noted for this group of subjects and subjects of approximately the same age range without hypertension (line 1) is the significantly increased cerebral vascular resistance ($P < .01$) in the hypertensive group.

In line 3 the findings in fifteen young subjects with hypertension and cerebral thrombosis are presented. All subjects were alert and cooperative at the time of the study. The cerebral infarctions were both recent and old, and in some cases, multiple. The duration of hypertension varied considerably. In some instances the blood pressure had been recorded as being higher than noted at the time these studies were made. The mean values for cerebral blood

vascular resistance is significantly higher ($P < .05$) than in subjects with hypertension alone.

In line 4 the results of eighteen determinations in thirteen alert normotensive elderly subjects are given. Most of these subjects showed evidence of systemic arteriosclerosis but none demonstrated clinical evidence of focal or generalized cerebral vascular insufficiency. The mean values for cerebral blood flow and metabolic rate are significantly lower ($P < .05$) than noted for younger alert normotensive subjects (line 1). The mean cerebral vascular resistance is higher, but not significantly greater, in the elderly group of subjects. The findings noted for cerebral blood flow, cerebral oxygen consumption and cerebral vascular resistance in this group of subjects are somewhat higher than values previously reported by us. On re-examination of our former data it was noted that some of the

subjects, though alert, had either hypertension or evidence of focal cerebral vascular disease.

The mean values of seventeen determinations in seventeen elderly alert hypertensive subjects are presented on line 5. Except for the significantly higher cerebral vascular resistance ($P < .01$) in this group of subjects, the values for cerebral blood flow and cerebral oxygen consumption are not significantly different from those noted for elderly alert subjects without hypertension (line 4). It should be noted, however, that the subjects with hypertension were considerably younger than the normotensive group. If one compares these results with values noted for subjects of a similar age range with hypertension and cerebral thrombosis (line 7), the cerebral blood flow and metabolic rate are significantly lower ($P < .01$) in the latter group of subjects.

Line 6 presents nineteen observations in thirteen normotensive elderly subjects with cerebral thrombosis. The cerebral infarctions were both old and recent, and some of the subjects were confused. The mean value for cerebral blood flow in this group of subjects is significantly less ($P < .02$) and that for cerebral vascular resistance significantly greater ($P < .02$) than noted for somewhat older subjects without clinical evidence of cerebral vascular insufficiency (line 4). There is no significant difference between the cerebral metabolic rate in these two groups of subjects.

In line 7 the mean values of fifty-eight determinations in fifty-one elderly subjects with hypertension and with cerebral infarction are given. The mental status of the subjects varied from rational to disoriented; none were comatose. The mean values for cerebral blood flow and metabolic rate are significantly lower ($P < .01$) than findings noted for subjects of approximately the same age range with hypertension alone (line 5). If one compares the findings for this group of subjects with those for somewhat older normotensive subjects with cerebral thrombosis (line 6), the only significant difference is the higher cerebral vascular resistance in the hypertensive group.

Line 8 presents sixteen observations in thirteen normotensive elderly senile subjects (senile psychosis and/or psychosis with cerebral arteriosclerosis). Except for the mental deterioration there was no overt evidence for cerebral vascular disease. There is a significant reduction of cerebral blood flow ($P < .05$) and of cerebral

metabolic rate ($P < .01$) and a significant increase of the cerebral vascular resistance ($P < .05$) when these values are compared with those of subjects of approximately the same mean age but without mental deterioration (line 4).

Line 9 presents fourteen observations in

TABLE II
EFFECT OF AGING ON CEREBRAL HEMODYNAMICS
AND METABOLISM

No. of Observations	Age Range	Mean Age	MAP	CBF	CVR	CMRO ₂	Ref. No.
1	4	17-18	17	97	79.3	1.3	3.6 *
2	19	18-36	..	85	65.3	1.3	3.8 12
3	25	20-44	29	91	52.0	1.8	3.1 24
4	12	18-40	30	91	53.0	1.8	3.4 11
5	12	18-47	32	94	57.5	1.7	3.2 *
6	15	38-55	..	96	60.5	1.6	4.0 12
7	23 *	45-75	56	101	46.0	2.2	2.9 24
8	17	56-79	63	97	50.6	2.0	3.3 12
9	23	45-86	68	95	46.0	2.1	2.7 11
10	13	57-99	80	94	47.7	2.1	2.7 *

* Data presented in this paper.

fourteen elderly subjects with hypertension and with psychosis due to senility or cerebral arteriosclerosis, or both. With the exception of the significantly increased cerebral vascular resistance ($P < .01$), the findings for this group of subjects are not significantly different from those noted for senile subjects without hypertension (line 8).

COMMENTS

I. Influence of Aging on Cerebral Hemodynamics and Metabolism. Table II presents the mean values of the results obtained in the present study, as well as those of other investigators, on the effects of aging on cerebral blood flow, vascular resistance and metabolism. The data are arranged in chronologic order. Since they are insufficient to indicate the changes occurring in each decade of life, they can only be divided into three groups: (1) below twenty years of age, (2) between twenty and fifty years of age and (3) over fifty years of age. All the subjects were presumably normal and showed no clinical evidence of either focal or generalized cerebral vascular insufficiency. Accurate psychologic evaluation was not carried out except in a few instances.

It is recognized that the data on four subjects under twenty years of age are not conclusive and are merely presented to give some idea of what may be expected in this age group. It may well be that during the developmental period the

oxygen and blood flow requirements of the brain are greater than during any other time of life. The data of most investigators demonstrate a significant reduction in the rate of cerebral blood flow and a fall of the cerebral metabolic rate in subjects over fifty years of age when compared to values noted for subjects between approximately twenty and fifty years of age. An examination of the data from which Shenkin¹¹ concludes that "aging per se has little effect on cerebral blood flow and metabolism" reveals that three of his subjects had focal evidence of cerebral vascular disease (E. G., W. H., B. B.) and in two subjects (J. P. and E. L.) the cerebral blood flow values were unusually high (98 and 80 cc./100 gm. of brain/min., respectively). The value for the cerebral metabolic rate reported for subject J. P. of 6.4 cc./100 gm./min. also seems somewhat high. If these five subjects are excluded from his data, a mean value for cerebral blood flow of 42 and for cerebral metabolic rate of 2.4 cc./100 gm./min. is obtained, findings that may be significantly different from the values noted in his younger control group. Although the values reported by Scheinberg¹² are somewhat higher than those reported by other investigators, his data nevertheless demonstrate a significant decrease of cerebral blood flow with aging and a fall in the cerebral metabolic rate.

The finding of a decreasing rate of cerebral blood flow and an increasing cerebral vascular resistance with advancing age suggests a progressive increase of arteriosclerotic involvement of the cerebral vessels. It is also evident that significant reductions in the rate of total cerebral blood flow from "normal values" may occur without obvious impairment in the functional activity of the central nervous system. In this regard, it may be of interest to mention that we have been able to reduce the rate of cerebral blood flow acutely from normal values to approximately 40 cc./100 gm./min. without a significant change in the cerebral metabolic rate and without obvious functional disturbances.¹³ The critical rate for cerebral blood flow at which signs and symptoms of cerebral ischemia became manifest in these acute studies was approximately 30 cc./100 gm. of brain/min. Even at this level in these acute studies the total cerebral metabolic rate did not change appreciably. Apparently in alert elderly subjects, despite a significant reduction of the total cerebral blood flow, adequate amounts of

oxygen and other essential substrates are being delivered to and extracted by the cerebral cells.

Cerebral arteriosclerosis is not relentlessly progressive in all individuals. In our studies on subjects over ninety years of age it was not unusual to find cerebral blood flow values within

TABLE III
EFFECT OF HYPERTENSION ON CEREBRAL HEMODYNAMICS
AND METABOLISM

	No. of Observations	Age Range	Mean Age	MAP	CBF	CVR	CMRO ₂	Ref. No.
1	8	28-49	42	152	57.0	2.7	3.4	*
2	6	38-56	44	136	52.0	2.6	3.1	11
3	13	34-54	45	159	54.0	3.0	3.4	16
4	6	41-68	61	139	39.0	3.4	2.4	11
5	17	52-84	68	150	48.7	3.2	2.8	*

* Data presented in this paper.

normal limits or only slightly reduced.¹⁴ Recently we had occasion to study the cerebral hemodynamics and metabolism of a subject presumably 105 years of age. He was alert, well oriented and completely self-sufficient. Considering his background, education and occupation, it was estimated that he had suffered little loss in mental acuity throughout the years. The values for cerebral blood flow, cerebral vascular resistance and cerebral oxygen consumption were 46.7, 2.4 and 2.7, respectively. Our studies on subjects who have survived beyond the expected life span suggest that the cerebral cells are fairly resistant to aging processes and that their relatively early deterioration may be primarily the result of vascular insufficiency.

II. Influence of Hypertension on Cerebral Hemodynamics and Metabolism. The composite data on the effects of hypertension on cerebral hemodynamics and metabolism are presented in Table III. Unfortunately, information regarding the duration of the hypertension is not available for most subjects. The subjects were all alert and showed no evidence of either focal or generalized cerebral vascular insufficiency. Subjects with malignant hypertension were not included in our series of cases.

There is good agreement regarding the effects of hypertension alone on subjects between twenty-eight and fifty-six years of age. Its only effect is to increase significantly the cerebral vascular resistance, a finding that, as Kety has previously suggested,^{15,16} may indicate participation of the cerebral vessels in the vasoconstriction associated with hypertension.

The cerebral vascular resistance which measures the frictional opposition to the flow of blood through the cerebral vessels may be influenced by changes in the viscosity of the blood or by increases or decreases of cerebral vascular tone. In these younger subjects the increased cerebral vascular resistance is believed to be due to an increased cerebral vascular tone associated with the hypertension. The values are significantly higher than obtained for normotensive subjects of a similar age range. A reduction of pressure in subjects with essential hypertension is associated with a return toward normal of the cerebral vascular resistance,^{13,17} indicating relaxation of the cerebral vessels. It is recognized that some are reluctant to ascribe to the cerebral vessels the capacity to increase their tone.^{18,19} It is not the purpose of this paper to attempt to refute this concept. It should be mentioned, however, that the reduction in cerebral blood flow associated with hyperventilation,²⁰ the administration of aminophylline²¹ and on breathing 10 per cent oxygen²⁰ is best explained at the present time by a concomitant increase of cerebral vascular tone.

Our findings in elderly alert hypertensive subjects (line 5) are essentially the same as noted for normotensive subjects (line 4) of approximately the same age range except for the higher cerebral vascular resistance in the hypertensive subjects. In six subjects without a history of a cerebral vascular accident or evidence of mental deterioration, Shenkin¹¹ observed a significant reduction of both cerebral blood flow and metabolism and a significant increase in cerebral vascular resistance when compared with values for normotensive subjects (line 4). He concluded from his observations that "aging and arteriosclerosis unaccompanied by hypertension and hypertension unaccompanied by arteriosclerosis do not reduce significantly cerebral blood flow and metabolism." It is generally recognized that arteriosclerosis may be regional in its distribution and that its presence elsewhere about the body may not necessarily reflect the degree of arteriosclerotic involvement of the cerebral vessels. We have studied the cerebral blood flow and metabolism of subjects with such marked arteriosclerosis obliterans that either unilateral or bilateral amputations have been necessary and obtained values not significantly different from those noted for control subjects. The fact that Shenkin's subjects had neither focal nor generalized cerebral vascular insufficiency would

seem to suggest the presence of only a moderate degree of cerebral arteriosclerosis. We cannot understand why, if in younger subjects hypertension is without effect on cerebral blood flow and metabolism, it should be expected to reduce these functions in older subjects without overt evidence of cerebral vascular disease.

The increased cerebral vascular resistance of elderly hypertensive subjects is most likely due to degenerative vascular disease and to the increased vascular tone associated with the hypertension. One would surmise that all cerebral vessels are not equally affected by the degenerative process and that the less involved vessels may perhaps undergo the greatest increase of tone when hypertension is present. One wonders if the increase in tone of the less involved vessels may not serve a useful purpose and assure those cerebral cells dependent for their blood supply on relatively rigid vessels of an adequate delivery of essential substrates.

It has been pointed out that a reduction of mean arterial pressure in elderly subjects with cerebral arteriosclerosis may in some cases be hazardous and result in transient episodes of cerebral ischemia.^{5,22} An acute reduction of mean arterial pressure in such subjects associated as it is with a relaxation of cerebral vascular tone and a reduction in total cerebral blood flow may result in a relatively greater degree of cerebral ischemia in the more rigid arteriosclerotic vessels because of a shunting of the available blood to the relaxed vessels. Preliminary studies²³ indicate that even a gradual reduction of pressure in elderly hypertensive subjects with cerebral arteriosclerosis may be hazardous even though there may be no significant change in the rate of total cerebral blood flow.

III. Effects of Cerebral Vascular Insufficiency (Focal or Generalized). The data on subjects with focal and/or generalized cerebral vascular insufficiency are presented in Table IV. Whenever possible, the pathologic nature of the cerebral vascular accident is given as well as the mental status of the subjects at the time of the studies.

It would appear that in subjects with cerebral vascular accidents and/or generalized cerebral vascular insufficiency, for which causes other than cerebral arteriosclerosis have been excluded, there is a significant reduction in both cerebral blood flow and metabolic rate. Our findings in young subjects (mean age forty-four years)

with hypertension and vascular insufficiency (line 1) are similar to those obtained by Shenkin (line 2) on subjects of approximately the same age (mean age fifty-one). Our observations suggest that cerebral thrombosis occurring in relatively young subjects is associated with

motor manifestations. Of the seventeen patients studied nine were known to have hypertension. In this group of subjects there was a significant decrease of both the cerebral blood flow and metabolic rate when compared to control subjects of the same age range. There is good agree-

TABLE IV
EFFECT OF VASCULAR DISEASE ON CEREBRAL HEMODYNAMICS AND METABOLISM

Condition	No. of Observations	Age Range (yr.)	Mean Age (yr.)	MAP	CBF	CVR	CMRO ₂	Mental Status	Ref. No.
1 Hypertension with cerebral thrombosis	15	34-49	44	138	41.6	3.4	2.5	Alert	*
2 Hypertension with cerebral thrombosis	7	45-60	51	131	37.0	3.7	2.6	Alert or disoriented	11
3 Hypertension with acute cerebrovascular disease	22	38-74	59	120	40.0	3.1	2.7	Alert or disoriented	24
4 Hyper- and normotension with chronic cerebrovascular disease	17	46-84	64	114	35.0	3.3	2.1	Alert or disoriented	24
5 Hypertension with cerebral thrombosis	51	50-101	65	137	40.5	3.6	2.3	Alert or disoriented	*
6 Psychoses: cerebral arteriosclerotic and senile	10	64-81	72	121	41.0	3.0	2.8	Psychotic	25
7 Normotension with cerebral thrombosis	13	53-88	73	97	38.1	2.7	2.5	Alert or disoriented	*
8 Psychoses with hypertension	14	55-98	75	148	41.6	3.8	2.4	Psychotic	*
9 Psychoses with normotension	13	72-92	83	94	38.4	2.6	2.2	Psychotic	*

* Data presented in this paper.

generalized cerebral vascular disease, and the infarction may merely represent a more advanced degenerative process in the involved vessel.

Heyman and Patterson²⁴ (line 3) studied cerebral hemodynamics and metabolism of subjects with acute cerebral vascular accidents. Twenty-one of their twenty-two patients with an acute cerebral vascular accident (mean age fifty-nine) had hypertension; five were rational and the remaining were either unconscious, had sensory manifestations or were aphasic. The studies were done within one to ten days after the onset of acute symptoms. The cerebral blood flow was significantly reduced when compared to their control subjects of approximately the same age, while the cerebral metabolic rate was only slightly lower. Twelve of their subjects with chronic cerebral vascular accidents (mean age sixty-four) had recurrent accidents (line 4), and the remaining five showed progressive mental deterioration without overt sensory or

ment between our results and those of Heyman and Patterson²⁴ (lines 4 and 5).

The results presented on lines 7 and 9 of Table IV indicate that significant reduction of both cerebral blood flow and metabolism may occur in subjects with cerebral arteriosclerosis, without coexistent hypertension. One cannot be absolutely certain, however, that our normotensive subjects with focal and/or generalized cerebral vascular insufficiency may not at some time have had hypertension.

The fact that there are no significant differences, except for cerebral vascular resistance, between normotensive and hypertensive subjects with or without cerebral vascular insufficiency is particularly interesting. It is also evident from the data that the mean age of subjects with hypertension in the various groups is always somewhat lower than for normotensive subjects. This would suggest that hypertension in some instances may well be an accelerating factor insofar as cerebral vascular disease is concerned;

however, the possibility remains that it may be the result of and not primary in its etiology. It is recognized that important variables, such as the duration of hypertension and its intensity, have not been controlled. Such factors as lipoprotein distribution, cholesterol level and cholesterol-phosphorus ratios, as well as the inherent susceptibility of the vascular wall have also not been considered.

IV. Cerebral Vascular Insufficiency and Mental Status. The mental deterioration frequently associated with aging has been attributed to cerebral vascular insufficiency. There can be no doubt that attendant with diminished energy production by cerebral cells there may be associated functional disturbances. Unfortunately, there is little evidence to indicate to what extent the cerebral metabolic rate can be chronically reduced before mental aberrations become apparent and if the symptomatology so produced simulates the changes characteristic of psychoses with cerebral arteriosclerosis.

In elderly subjects with psychosis and cerebral arteriosclerosis there is a significant reduction in both cerebral blood flow and metabolism. In our studies we were unable to differentiate subjects with psychosis due to cerebral arteriosclerosis from those with senile psychosis. Freyhan and Kety²⁵ (Table IV, line 6) have previously reported similarly low values for cerebral hemodynamics and metabolism in both groups of subjects. On reviewing the data, it may be seen that equally low values are reported for presumably alert subjects. (Table IV, line 1.) It should again be pointed out that the procedure used estimates total cerebral blood flow and oxygen utilization so that the extent of vascular insufficiency in any specific area of the central nervous system cannot be estimated. It may be that in subjects with psychosis due to cerebral arteriosclerosis the vascular insufficiency may be most marked in the frontal regions; whereas in alert subjects with a reduction of cerebral blood flow and oxygen consumption of a similar magnitude, the degree of vascular deterioration may be least in these areas. The increased sensitivity of the higher brain areas to oxygen lack and to central nervous system depressant drugs is well-recognized. This increased susceptibility is believed to be due to their greater metabolic demands. Thus if there was marked arteriosclerosis of the larger cerebral vessels, i.e., internal carotids limiting the blood flow to the brain, one might expect that changes of func-

tional activity would be most marked in the higher centers. If these hypotheses are correct, it may be possible to explain the dementia of certain elderly subjects on the basis of an inadequate delivery of essential substrates to the cerebral cells. The fact that in senile dementia there may be marked deterioration without a significant degree of cerebral arteriosclerosis suggests that in such cases the primary disturbance may be intracellular, and the decrease in cerebral blood flow may be secondary to the decreased metabolic demands of the cerebral cells.

SUMMARY AND CONCLUSIONS

The influence of aging, hypertension and focal and/or generalized cerebral vascular disease on cerebral hemodynamics and metabolism was determined, and the results obtained were compared with those of other investigators.

Most authors are in agreement that aging is associated with a significant decrease of the rate in cerebral blood flow, a fall in the cerebral metabolic rate and a significant increase in cerebral vascular resistance. These changes are most likely due to a progressive increase in the degree of cerebral arteriosclerosis with age. Significant reductions in the rate of cerebral blood flow from values found in normal young subjects may occur in elderly subjects acutely and chronically without a significant change in cerebral oxygen utilization and without obvious impairment in the functional activity of the higher centers.

Essential hypertension in both young and elderly subjects is invariably associated with an increased cerebral vascular resistance. In elderly subjects the increased cerebral vascular resistance is due in part to cerebral arteriosclerosis.

In subjects with essential hypertension without overt evidence of cerebral vascular insufficiency (focal and/or generalized), the rate of cerebral blood flow and oxygen utilization is not significantly different from values noted for subjects of a similar age range without hypertension. Cerebral vascular insufficiency may occur earlier in subjects with hypertension but the reduction in cerebral blood flow and metabolism is not of greater magnitude than noted for normotensive subjects with similar disturbances. Those results suggest that hypertension may well be an accelerating factor in the production of cerebral vascular disease but is not necessarily etiologic.

In elderly subjects with psychosis (senile and/or cerebral arteriosclerosis) with or without

hypertension and no focal evidence of cerebral vascular insufficiency, a significant reduction of cerebral blood flow and cerebral oxygen consumption was noted. One cannot conclude with certainty that the mental deterioration in such subjects is a consequence of the decreased delivery of essential substrates.

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Seminars on the Hemolytic Anemias

Hereditary Spherocytosis*

LAWRENCE E. YOUNG, M.D.

Rochester, New York

HEREDITARY spherocytosis, the hemolytic disorder to be considered in this paper, is inherited as a mendelian dominant and is favorably affected by splenectomy in practically all cases despite persistence of structural and metabolic abnormalities of the red cells. This paper will deal chiefly with existing knowledge of the red cell abnormalities and their probable relationship to the hemolytic effect of the spleen in these patients. The clinical and laboratory findings, genetics and therapy will be discussed in the light of our present understanding of the cellular abnormalities.

Vanlair and Masius¹ in 1871 provided the first accurate description of this disease but the merit of their observations was not recognized until recently.² Dacie³ has written the most recent and complete summary of both the clinical and the laboratory aspects of hereditary spherocytosis (hereafter abbreviated as HS) and has given a detailed account of the major contributions to our understanding of this condition. He has quite properly emphasized the fact that hereditary spherocytosis is but one of a number of distinct types of hereditary hemolytic disease. The other recognized types are Mediterranean anemia, hereditary elliptocytosis, certain congenital non-spherocytic hemolytic anemias, sickle cell anemia and other hemolytic disorders associated with abnormal hemoglobin.

Among persons of English or northern European descent, hereditary spherocytosis is the most common of the hereditary hemolytic disorders which are now recognized. Since 1946 fifty cases of chronic spherocytosis, nearly all of which would qualify as examples of HS, have been studied in the Strong Memorial Hospital. These patients have been drawn from twenty-eight families in an area of upstate New York

containing a population of approximately one million people. Some HS cases in this area have no doubt gone unrecognized and presumably some well documented cases in the area have not been investigated in our clinic. The figures cited may nevertheless permit a rough estimate as to the frequency of this disease among persons descended very largely from European ancestors. There are only four reports of HS among American Negroes³ and no data in published reports which would enable one to estimate the incidence of this disorder in non-European stocks. Dacie³ states that it cannot be considered a rare disease in England, and he points out that with low mortality and excellent results of splenectomy the incidence is likely to increase if there is a steady mutation rate. This disease apparently causes little or no decrease in fertility.⁴

DEFINITION OF SPHEROCYTOSIS

Spherocytes may be defined as red cells having a thickness:diameter ratio significantly greater than that of normal (human) red cells.⁵ All degrees of "sphering" are encountered in blood from HS patients; some of the cells, although abnormally thick, are nevertheless biconcave, while others may be nearly spherical. The thicker cells can be recognized in well prepared blood smears by their lack of the area of relative translucency, the central "pallor," seen in the center of normal cells. (Fig. 3.) The diameter and the ratio of surface area to volume of spheroidal cells are less than normal, while the mean corpuscular volume is normal⁶ but may be increased in cases with reticulocytosis. Ponder⁷ stresses the point that spherocytes should be distinguished from "spherical forms," the latter being produced *in vitro* by various means and

* From the Department of Medicine, The University of Rochester School of Medicine and Dentistry, and the Medical Service of the Strong Memorial Hospital, Rochester, New York. The studies of the author and his associates referred to in this seminar were aided by contracts between the University of Rochester and the Office of Naval Research, Department of the Navy, and the Public Health Service.

exhibiting characteristics different from those of most spherocytes produced *in vivo*.

The term spherocyte was apparently first used by Christophers and Bentley⁸ rather than by Naegeli⁹ who has often been credited with introduction of this term. Naegeli regarded the spherocyte as pathognomonic of familial acholuric jaundice (HS), despite the fact that Vanlair and Masius¹ had observed microcytes (spherocytes) in heated blood and Banti¹⁰ in 1913 had increased the osmotic fragility of red cells by the action of immune serum. Dameshek and Schwartz in 1938 reported production of spherocytes in guinea pigs by intravenous injection of immune rabbit serum and described three cases of acute spherocytic hemolytic anemia associated with circulating hemolysins.^{11,12} As a result of these observations they emphasized the fact that spherocytosis is by no means confined to the hereditary human disorder.

CLINICAL COURSE

Anemia. The degree of anemia in hereditary spherocytosis varies considerably from patient to patient, but is rarely severe. In most cases the rates of red cell production and destruction are sufficiently balanced so that only minor fluctuations in hematocrit and hemoglobin concentration are noted during long periods of observation prior to splenectomy.^{3,13,14} Both minor and major crises occur from time to time in HS patients, sometimes simultaneously in more than one member of a family, especially in association with infectious diseases. During such periods there may be evidence of accelerated red cell destruction beyond the usual rate, and in a few cases rapid destruction of normal donated red cells has been reported,^{15,16} suggesting the development of an "extracorporeal" hemolytic mechanism. Although, the direct antiglobulin (Coombs) test is negative in most cases¹⁷ (in all but one in our series), positive reactions have been reported in a few instances^{18,19} especially during periods of accelerated hemolysis. Auto-antibodies like those encountered in acquired hemolytic anemia have also been demonstrated in the serum during crisis.¹⁹ Major crises may at times be associated with a sudden decrease in erythropoiesis due to aplasia²⁰ or maturation arrest¹⁶ in the bone marrow. Severe anemia develops rapidly when marrow failure is added to the chronic hemolytic state.

Bile Pigment Metabolism. The serum bilirubin concentration may fluctuate considerably in HS

cases prior to splenectomy. The range of total serum bilirubin figures in our group of patients was from 0.4 to 13.0 mg. per 100 ml. with a mean figure of approximately 2.0 mg. The patients frequently note malaise, anorexia and mild fever in association with bouts of jaundice. In some of these episodes cholecystitis may be an important contributing factor. Cholelithiasis develops in a large share of HS patients who are not splenectomized before the second decade of life.¹⁸ Bates and Brown²¹ found calculi in only one of nineteen adequately investigated children with HS under age ten, but in their series of seventy-four patients over age ten, thirty-nine had gallstones. Fecal urobilinogen excretion is usually substantially increased, the average figure being about six times normal in Watson's experience.²² Watson found that urinary urobilinogen excretion was usually within the normal range except during periods of crisis or in the presence of complications adversely affecting liver function. Bilirubin is not ordinarily found in the urine (acholuria) in this disease, as in other hemolytic disorders, but may appear if the patient suffers complications such as hepatitis or obstruction of the common bile duct by a gallstone.

Since either jaundice or anemia or both may be lacking a good share of the time in many HS patients, the terms "congenital hemolytic anemia," "familial acholuric jaundice," etc. are clearly less appropriate than "hereditary spherocytosis," the designation for this disease which has become widely accepted in recent years.

Associated Abnormalities. Gänsslen, Zipperlen and Schüz,²³ and Hansen and Klein²⁴ frequently encountered various congenital abnormalities such as tower skull, polydactyly and infantilism in HS patients. Hypogonadism has been reported in HS patients by several authors.³ Bernard and associates²⁵ have described their findings in a remarkable family of thirteen affected children, nine of whom were retarded both mentally and physically. All of the children, including the four regarded as infantile, showed marked improvement in mental and physical development after splenectomy. In the experience of most observers, however, associated abnormalities have been uncommon. In his study of 183 HS patients in London, Race⁴ reported cervical ribs in one patient, congenital absence of a hand in another and mental deficiency in a third. We have encountered skeletal abnormalities in only one patient, a boy whose parents refused to

permit splenectomy until he was thirteen years of age. By that time the bones forming the cranial vault showed roentgenographic thickening and physical development was retarded. In another family studied in this clinic, all three affected members suffer from otosclerosis, an associated abnormality also reported by Gänsslen et al.²³

We have thus far noted no ulcers of the skin of the lower legs in our HS patients. This complication, frequently involving both legs, has been described in a number of cases in the literature but the incidence is difficult to estimate.³ The pathogenesis of such ulcers is obscure since there is no reason to postulate vascular occlusion by the red cells in this disease, as there is in sickle cell anemia.

INHERITANCE

The studies of Race,⁴ Meulengracht²⁶ and others^{3,13} indicate that hereditary spherocytosis is probably inherited as a mendelian dominant. One-half of the offspring of an affected parent are similarly affected. In the families studied by Race⁴ as well as in those studied in our clinic,¹³ however, there is a significant shortage of affected siblings of propositi (24 per cent instead of the expected 50 per cent incidence). We have, moreover, encountered five patients whose parents on repeated testing had no demonstrable hematologic abnormality. Normal findings were also obtained with blood samples from all other available relatives of these five patients, with one highly significant exception. An infant sibling of one of the patients was found to have hematologic abnormalities like those of HS, a finding which suggests that one of the parents carries the gene for HS but the gene penetrance or expressivity is such as to be undetectable by available methods (? "carrier" state). The same may be true in each of the other four of our families and in the four families described by Race⁴ and one family reported by Meulengracht,²⁶ in which both parents of the propositus appeared to be unaffected. Other possibilities are gene mutation in the propositus, illegitimacy (considered very unlikely), and occurrence of an HS-like disease that is either non-hereditary or at least not inherited as a mendelian dominant. None of our five patients in this group has yet produced offspring for study. All of these five patients have been splenectomized with excellent response despite persistence of red cell abnormalities as in typical HS cases. Further investigation

of these families in the years to come should yield results of interest.

The homozygous form of HS has not yet been demonstrated with certainty, although its occurrence has been suspected in families studied by Race⁴ and Bernard et al.²⁵ Since practically all HS patients have the heterozygous form of the disease, the term "spherocytic trait," which is sometimes used in speaking of mildly affected persons, requires definition. Gänsslen and associates,²³ Campbell and Warner,²⁷ Wiedemann,²⁸ Discombe²⁹ and others¹³ have described very mildly affected patients, sometimes encountered in family groups. Although the disease tends to remain mild in such persons, crises may nevertheless occur, as in one of our patients whose red cell count dropped from 5.3 to 2.7 millions with 10 per cent reticulocytes during a bout of infectious mononucleosis.¹³ The life span of HS cells is probably much less than normal even in the non-anemic, non-icteric patients,³⁰ and even such patients probably develop pigment gallstones much more readily than the rest of the population. In view of these probabilities and the fact that even the most severely affected HS patients are heterozygotes, it seems advisable to avoid use of the term "spherocytic trait," lest it be thought that this term denotes a condition somewhat comparable to sickle cell trait.

NATURE OF THE ERYTHROCYTE ABNORMALITIES

The fact that spherocytosis persists after splenectomy in HS cases, despite permanent relief from anemia, has long been cited as evidence that the red cells, rather than the spleen, are at fault. Further evidence that these red cells are abnormal has been accumulated during transfusion experiments. Dacie and Mollison³¹ showed that normal red cells survived normally after transfusion to HS patients, while HS red cells taken either before or after splenectomy disappeared rapidly from the circulation of a normal recipient. Schrumpf,³² moreover, demonstrated nearly normal survival of HS red cells after transfusion to a splenectomized recipient, an observation supporting the hypothesis that abnormalities of the HS erythrocyte are of little consequence once the spleen is removed from the circulation.

The pathogenesis of the abnormal shape of the red cells in hereditary spherocytosis has been a subject of considerable interest and controversy. The nucleated red cells in the bone marrow of

HS patients have normal shape³ and the reticulocytes are either normal in shape or only slightly less discoidal than reticulocytes in normal blood.³³ Sphering apparently occurs in varying degrees after the red cells have matured in the circulation. In 1910 Troisier³⁴ suggested that sphering and increased osmotic fragility of the red cells might be due to the action of hemolysins and in 1913 Banti¹⁰ concluded that such hemolysins might be produced chiefly by the spleen. The previously cited observations of Dameshek and Schwartz^{11,12} supported the concept of erythrocyte damage by hemolysins, and these authors suggested that circulating hemolysins might be responsible for spherocytosis in the hereditary disorder as well as in acquired hemolytic anemia. On the other hand, Naegeli⁹ and more recent investigators³ have held the view that in the hereditary form of spherocytosis the red cells are abnormal when delivered from the marrow, even though sphering may not be evident until the cells are mature.

Erythrocyte Fragility. *Osmotic fragility:* Since Chauffard³⁵ first demonstrated increased osmotic fragility of red cells from HS patients in 1907, numerous observers have confirmed his findings. Haden,³⁶ and subsequently Castle and Daland,³⁷ and Dacie and Vaughan,³⁸ correlated osmotic fragility with the degree of sphering of the red cells and concluded that the spherocyte could absorb less water from hypotonic media than could normal biconcave cells before undergoing lysis. Castle and Daland,³⁷ Guest³⁹ and Izzo and Young¹⁴ have shown that HS cells behave osmotically in the same manner as normal cells; that is, the increments in the cell volume with each decrement in tonicity of the medium are the same for HS and normal cells. HS cells apparently undergo lysis in hypotonic media, not because of anomalous swelling but because their capacity to swell is limited by their smaller surface area.⁶

Results of osmotic fragility tests have been recorded in a variety of ways, each of which has certain advantages and disadvantages.³ Probably the most commonly used method of expressing results quantitatively is that of plotting the per cent hemolysis in each tube of the series of hypotonic salt solutions against the corresponding concentration of salt in the tube. Representative curves obtained in this manner from HS patients are shown in Figure 1 and are similar to those published by Dacie.³ If large proportions of the red cells in the patient's

blood are markedly spheroidal, a large part of the fragility curve is shifted to the left of the normal range in such a graph. In cases with fewer spheroidal cells in the circulation, "tailed" curves are obtained. In still other cases very few spherocytes are found in the peripheral

HEREDITARY SPHEROCYTOSIS

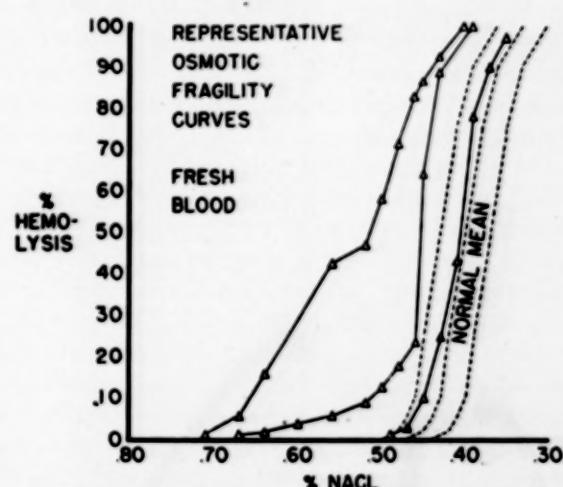


FIG. 1. Representative osmotic fragility curves obtained with freshly drawn blood from patients with hereditary spherocytosis. Area between broken lines shows the normal range ($\text{mean} \pm 20$) based on examination of twenty-six normal specimens. (Reproduced from paper by Young and Miller.⁴⁰ Courtesy of Lea & Febiger.)

blood and the fragility curves are within or very close to the normal range.

Emerson, Shen, Ham and Castle,⁴¹ and subsequently other observers,^{13,42-44} showed that HS cells undergo a considerably greater increase in osmotic fragility than do normal red cells when incubated twenty-four hours at body temperature under sterile conditions. In addition to the theoretical interest which this procedure carries, such tests may be helpful in detecting otherwise inapparent or questionable abnormalities of the red cells in suspected HS cases. In approximately one-fourth of the HS cases in this clinic the osmotic fragility of the freshly drawn cells was in or near the normal range, varying slightly from time to time. The fragility of the incubated cells, however, was clearly outside the normal range in each instance. Results in five representative cases from this group are shown in Figure 2.

After splenectomy the median corpuscular fragility (%NaCl causing 50 per cent lysis) of freshly drawn HS cells may increase slightly but

"tails" on the osmotic fragility curves are usually much smaller. A large portion of the osmotic fragility curve of incubated HS cells is nearly always more markedly increased after splenectomy than before.^{13,14} Dacie³ explains that two factors acting in opposite directions may affect

no matter how mild the clinical manifestations of the case or how long the interval since successful splenectomy. Neither the magnitude of the shift in median corpuscular fragility after incubation of the erythrocytes nor the shape of the fragility curve obtained with incubated cells, however,

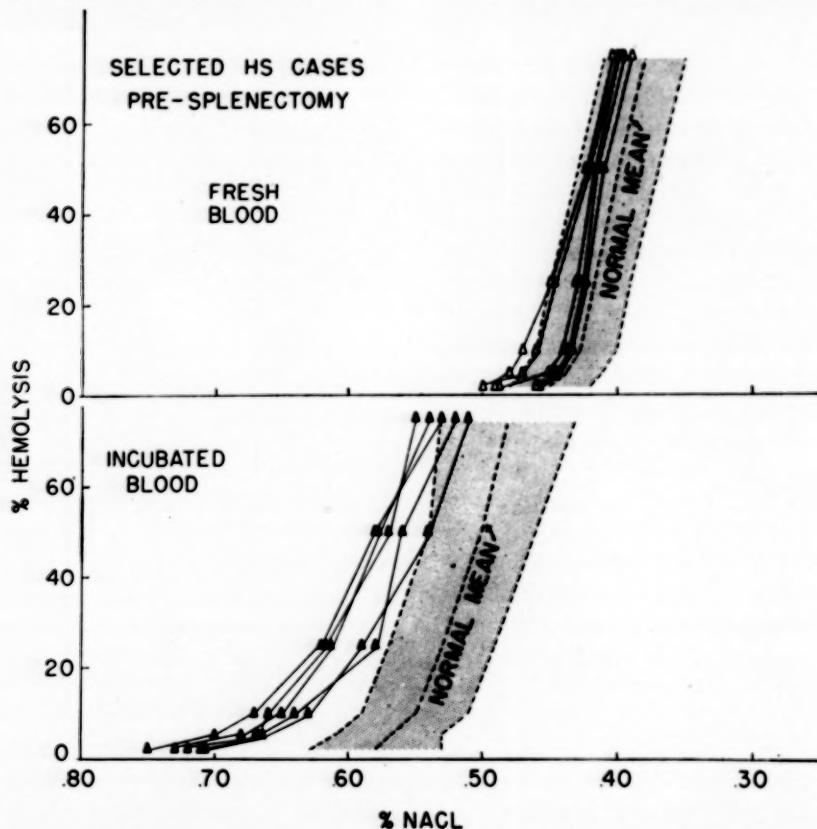


FIG. 2. Osmotic fragility curves obtained with freshly drawn and incubated blood from five selected patients with HS. All of the curves for normal blood are in or near the normal range, whereas all of the curves for incubated blood are clearly outside the normal range.

fragility after removal of the spleen: (1) removal of an organ capable of increasing the fragility of the abnormal red cells passing through it, and (2) longer survival of the abnormal red cells (which might make them more susceptible to the effects of incubation *in vitro*).

In general, the shift in osmotic fragility after incubation of the red cells has been less marked in other conditions associated with spherocytosis, especially in autoimmune hemolytic disease.⁴⁰ In the latter disorder the osmotic fragility of the incubated cells may be increased during phases of markedly accelerated hemolysis but is within normal limits during quiescent phases, even though the antiglobulin test often remains strongly positive. Incubated HS cells, on the contrary, nearly always show increased fragility

can be considered uniquely characteristic of hereditary spherocytosis. Spherocytes encountered in the blood of patients with myeloproliferative disorders, for example, may show changes in osmotic fragility on incubation that are similar to those observed with HS cells.¹⁴

Mechanical Fragility. Shen, Castle and Fleming⁴⁵ in 1944 reported that spherocytes, as well as sickled and agglutinated red cells, have abnormally great susceptibility to destruction by the trauma of glass beads in rotating flasks. Using the method of these authors, we found that the mean per cent hemolysis, or mechanical fragility (M.F.), of the red cells in pre-splenectomy HS cases was four to five times normal and the mean M.F. of red cells from splenectomized HS patients was about three times the normal

mean.^{13,14} The differences between normal and HS cells after sterile incubation at 37°c. for twenty-four hours were less significant in the tests of mechanical fragility than in the tests of osmotic fragility. The persistence of increased mechanical fragility of freshly drawn HS cells after splenectomy, despite normal or nearly normal life span of the cells in the patient, poses interesting questions about the physiologic interpretation of the mechanical fragility test.

Still other types of fragility tests have been employed in studies on HS cells, in particular the estimation of resistance of the cells to the action of lysolecithin, a chemical hemolysin.^{3,13,46} Evaluation of these tests is difficult because of the limited observations reported from any one laboratory.

Autohemolysis during Incubation in vitro. Ham and Castle^{47,48} in 1940 described observations on hemolysis and increases in volume and osmotic fragility of normal and spheroidal red cells during sterile incubation at body temperature for periods up to thirty-two hours. They noted that swelling of spheroidal cells progressed at about the same rate as in normal cells but that hemolysis occurred after shorter periods of incubation. They concluded that the spherical shape was reached more quickly by the cells which were already thicker than normal before incubation. They also cited experiments showing that the changes observed in incubated red cells were not due to lytic substances present in or derived from the blood serum. These authors suggested that the observed changes might be due to metabolic processes leading to an increase in osmotically active constituents of the cells, and thus to progressive swelling and ultimately lysis. They further postulated that similar changes might occur in spheroidal cells while stagnating within the spleen and might largely account for accelerated red cell destruction in hemolytic disorders characterized by spherocytosis.

In 1941 Dacie⁴⁹ reported that HS cells underwent lysis five to ten times as rapidly as normal red cells when incubated at body temperature for forty-eight hours. Lysis was usually grossly apparent after twenty-four hours, as in the experience of Ham and Castle,⁴⁷ but was much more pronounced after forty-eight hours. He found that autohemolysis was reduced slightly when washed HS cells were suspended in autogenous serum previously heated to 56°c. for thirty minutes. During the second twenty-four-hour period of incubation marked lysis

nevertheless occurred after inactivation of heat-labile components of serum and was not inhibited by replacing autogenous serum with normal serum. These findings have since been confirmed by Caroli et al.⁵⁰ and in our laboratory.^{13,14,40}

Since 1949 we have measured autohemolysis in defibrinated blood by methods similar to Dacie's³ and have come to have a high regard for the test as a useful clinical laboratory procedure.⁵¹ We have thus far tested blood specimens from ninety normal persons, forty-five patients with HS or very similar disorders associated with chronic spherocytosis, twenty-five patients with autoimmune hemolytic disease, thirty-four with myeloproliferative disorders and forty-four patients suffering from various other types of blood dyscrasias. Autohemolysis is nearly always greater than normal in HS cases. It may be substantially increased in autoimmune hemolytic disease during very active phases of the illness when spherocytosis is marked but tends to be normal during relatively quiescent phases. Slight to moderate increases in autohemolysis have been noted in certain of the other patients, but only in HS have substantial increases been recorded in nearly all cases including the previously splenectomized patients.

Dacie³ has pointed out that red cells from patients with paroxysmal nocturnal hemoglobinuria also undergo lysis in this test more rapidly than normal. Unlike HS cells, however, PNH cells may show hemolysis within the first hour of incubation and lysis is accelerated if the pH is reduced and if the tests are carried out with clotted blood rather than defibrinated blood. Our experience agrees completely with Dacie's that abnormally rapid spontaneous lysis during the forty-eight hour period of sterile incubation may have several causes and that the test should be regarded as non-specific. When positive, it is nevertheless a "valuable pointer to a hemolytic process."³

Effect of sugars and nucleosides on autohemolysis: Selwyn and Dacie⁵² have shown that during incubation HS cells take in sodium at about the same rate as normal cells and lose potassium slightly more rapidly than normal cells. These changes, as well as hemolysis, occur much more slowly when glucose is added to the defibrinated blood to raise the initial glucose concentration to approximately 500 mg. per cent. They have also pointed out that after swelling during the first twenty-four hour period of incubation, both HS

and normal red cells shrink to approximately their original volume during the second twenty-four hour period, at which time the HS cells are usually undergoing rapid lysis. Selwyn and Dacie^{3,52} concluded that autohemolysis of HS cells is not due to progressive swelling of the cells⁴⁷ but may be related to a "defective cell membrane" which undergoes "degenerative irreversible shrinkage" more rapidly than normal.

Miss Izzo⁵³ has demonstrated in our laboratory that the thickness:diameter ratio increases more rapidly in HS cells than in normal red cells during incubation. Despite the fact that she found the rate of disappearance of glucose in incubated HS blood to be normal, the possibility of abnormalities of carbohydrate metabolism in HS cells was considered.^{40,53} Studies similar to those of Selwyn and Dacie⁵² were conducted and it was found that addition of either glucose or mannose to defibrinated blood markedly reduced autohemolysis of HS cells, while pyruvate had no such effect.⁵¹ These results were not surprising in view of Maizels' observation that shifts in cations and water in incubated normal red cells were largely prevented when the blood was incubated with glucose or mannose.⁵⁴ Maizels attributed his results to the fact that glycolysis provides energy needed to maintain the differences in cation concentration between the red cells and plasma. Altman and Izzo⁵⁵ showed that glycolytic inhibitors such as sodium fluoride and iodoacetate nullified the effect of glucose on autohemolysis of red cells from our HS patients as anticipated. They also found that addition of adenosine or guanosine to HS blood substantially reduced autohemolysis in most cases but to a lesser extent than did glucose and mannose. Since Dische⁵⁶ had shown that adenosine and guanosine undergo phosphorylotic cleavage to yield ribose-phosphate which in turn may be converted to triose-phosphate, it seemed possible that adenosine and guanosine reduced autohemolysis by providing metabolizable substrates for continued glycolysis.

Abnormalities of carbohydrate metabolism: Prankerd and Altman⁵⁷⁻⁵⁹ sought abnormalities of carbohydrate metabolism in the red cells from eighteen of our HS patients by using P³²-labelled orthophosphate to measure the rate of incorporation of P³² into the various organic phosphate esters. Heparinized blood samples were incubated at body temperature with

NaH₂P³²O₄ and at appropriate intervals (usually two and four hours) the phosphorus compounds were extracted with trichloracetic acid from samples of red cell stroma and the remaining intracellular material. Phosphorus-containing fractions were isolated from these extracts by two-dimensional paper chromatography and the relative specific P³² activities in each fraction were calculated.

The rate constant for P³² exchange between plasma and HS cells was found to be approximately the same as in normal blood. In the intracellular material from normal red cells studied by this method the relative specific activities (RSA's) of 2,3-diphosphoglycerate (2,3-DPG) and of adenosine triphosphate (ATP) exceed that of inorganic phosphorus (IP) but this relationship was found to be reversed in HS cells. It may be concluded from this finding that, whereas in normal cells ATP is the major source of intracellular IP, another mechanism is probably involved in production of IP in HS cells. Moreover, the RSA's of 2,3-DPG and ATP were found to be significantly lower than normal in most cases, indicating a slower flow of P³² into these phosphate esters. The abnormalities in phosphate partitioning revealed by these studies persisted after splenectomy.

Addition of adenosine to the HS cells incubated with NaH₂P³²O₄ altered the intracellular phosphate relationships toward normal in ten of eighteen cases. Investigations designed to explain the lack of the effect of added adenosine in some cases are currently in progress in our laboratory and may provide a basis for subdivision of the group of HS cases which now appears clinically and hematologically homogeneous.

Abnormalities of phosphate partitioning have been demonstrated in red cells from patients with spherocytosis accompanying other types of hemolytic disease, notably autoimmune hemolytic disease and myeloid metaplasia.⁵⁰ Only in HS cells, however, has the favorable effect of adenosine been observed. Further studies are obviously needed on the mechanisms underlying the metabolic disturbances in various types of abnormal cells.*

* The deficiency in potassium in HS cells^{52,60} might be expected to lead to a slower rate of regeneration of ATP by transfer of phosphate from phosphopyruvic acid to adenosine diphosphate (ADP), mediated by the potassium-requiring enzyme, pyruvic phosphoferase.^{58,59} This may account in part for the slower flow of P³² into organic phosphate esters in HS cells. The effect of adenosine may be due largely to conservation of ATP, since formation of

The RSA's of the phosphate esters in stroma of HS cells, as in the stroma of normal red cells, did not parallel those found intracellularly. Although the ATP pool in the stromal fraction of HS cells tends to be smaller than normal, the flux of P^{32} into ATP as well as into 2,3-DPG is so much smaller than normal that accurate measurements of radioactivity could not be made. With the data available, no relationship could be shown between the degree of spherling of the cells and stromal ATP content, which was low in most cases. It seems possible, nevertheless, that stromal ATP has some part in maintaining the biconcave shape of red cells.^{58,59}

Results of the more recent investigations of Prankerd and Altman suggest that inherited abnormalities of red cell metabolism may be largely responsible for the principal hematologic features of hereditary spherocytosis. The shape of freshly drawn HS cells and the increased spherling, fragility and autohemolysis after incubation may well be due to derangements in the energy-yielding reactions of glycolysis. Although it is still difficult to explain some of the clinical features of hereditary spherocytosis, especially the crises, on this basis it seems likely that degenerative changes occurring in HS cells trapped in the spleen are of prime importance in shortening the life span of these cells.

ROLE OF THE SPLEEN

The spleen is regularly enlarged in patients with hereditary spherocytosis, although in some cases it cannot be felt, especially in young children. When the freshly excised HS spleen is cut with a sharp knife, remarkably little blood oozes from the surface. This observation may be correlated with the microscopic findings in such spleens, the most prominent of which is the engorgement of the pulp with red cells, while the venous sinusoids are for the most part inconspicuous and contain few blood cells.^{3,14,61,62} Although in some areas of the fixed sections of HS spleens it is admittedly impossible to determine the exact site in which the red cells are collected,

ribose-phosphates and subsequently triose-phosphates from adenosine does not require ATP as does the formation of hexose-phosphate from glucose.⁶⁰ This mechanism of action of adenosine would apply only if sufficient ADP were available. An alternate possibility for explaining the action of adenosine is that adenosine might undergo phosphorylation directly to form adenylic acid which could then be converted to the corresponding di- and tri-nucleotides by means of an adenylate kinase.

it seems likely that stagnation and hemoconcentration occur largely in the pulp rather than in the sinusoids.

Whipple⁶³ postulated that spherocytes may be trapped in the pulp because they cannot pass as readily as normal discoidal cells through slit-like openings into the venous sinusoids. Dacie⁶⁴ perfused spleens from HS patients with saline introduced through the splenic artery and found that it took much longer to wash such spleens free of blood than in similar experiments with spleens from patients with other disorders. Emerson, Shen, Ham and Castle⁴¹ transfused HS patients with serologically identifiable normal erythrocytes prior to splenectomy and demonstrated much higher proportions of autogenous cells in the spleen than in peripheral venous blood at the time of splenectomy. The HS cells were not only selectively trapped in the spleen but were found to have much greater osmotic fragility than the HS cells in peripheral blood. On the other hand, the normal transfused red cells collected in the spleen, as well as the donor cells in the patients' peripheral blood, showed no increase in fragility. Similar observations have been made in our laboratory,^{42,61} and closely related experiments have been described more recently by Weisman, Hurley, Harris and Ham.⁶⁵

Figure 3A shows a smear prepared from capillary blood of one of our HS patients at the time of splenectomy, when 61 per cent of the red cells in peripheral blood were autogenous and 39 per cent donated. There was very little spherling and only slight increase in osmotic fragility of the autogenous cells in blood from the antecubital vein at that time. A smear of washed red cells prepared from the minced spleen of this patient is shown in Figure 3B. Of the red cells in this preparation, 84 per cent were identified serologically as autogenous and the autogenous cells were found to have markedly increased osmotic fragility. The donated red cells in the minced spleen, on the contrary, exhibited nearly normal fragility. It is therefore reasonable to conclude that the spherocytes, which are numerous in the smear of Figure 3B are largely if not entirely autogenous cells. The selective spherling effect of the spleen on HS cells is apparent from repeated experiments of this type.

After such effects of the spleen had been demonstrated, there remained the question as to whether the selective filtration of spherocytes was due to peculiarities of the HS spleen or to a

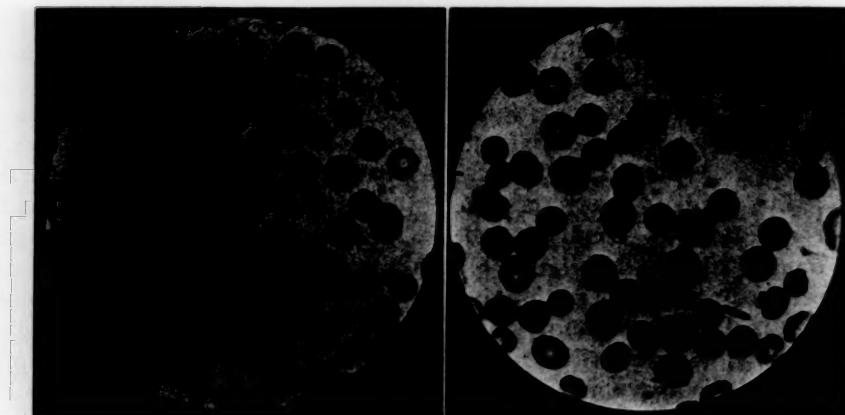


FIG. 3. A, photomicrograph of smear from capillary blood of transfused HS patient at time of splenectomy; $\times 970$. B, smear of washed splenic "minced" blood from same patient. (Reproduced from paper by Young, Platzer, Ervin, and Izzo.⁶¹ Courtesy of Grune & Stratton.)

sort of trapping which almost any human spleen might exhibit if presented with HS red corpuscles. In an effort to answer this question we prepared mixtures of normal red cells and HS cells from a splenectomized patient and perfused such mixtures through spleens freshly excised from patients with idiopathic thrombocytopenic purpura.⁶¹ In each of three experiments the HS cells were selectively removed from the mixture and after perfusion was stopped the HS cells were found to predominate in blood samples washed from the minced spleen. These experiments showed that spleens from patients with non-hemolytic disease were fully capable of selective trapping of HS cells. We are still uncertain, however, as to the exact mechanism of the trapping.

Although the length of the sojourn of red cells in the spleen is not known, the available evidence indicates that HS cells traverse the spleen slowly. Gibson and associates⁶⁶ have shown, moreover, that the hematocrit of blood within the spleen may be as high as 80 per cent. Stagnation and hemoconcentration within the spleen can be expected to cause a reduction in the supply of metabolizable substrate available to each red cell, and the limited supply is also utilized by the other cells of the spleen. It seems likely that the HS cell, because of its metabolic "handicaps," cannot maintain energy production adequately while caught in the unfavorable environment of the spleen. An attractive hypothesis is that deficiency in energy production may then lead to degenerative changes in the cell, increased spherling and ultimate lysis.^{58,69} Repeated visits to the spleen are presumably

required to bring about actual lysis of most HS cells.

Once the splenic trap is removed, substrate for energy production is apparently supplied so adequately in the active circulation that the HS cells maintain their viability despite the metabolic handicaps. Tentative analogies may be drawn between the effects of splenectomy and the effects of adding glucose, mannose, adenosine or guanosine to the HS cells which have been removed from the circulation and placed in a test tube. In either case, improved maintenance of viability of the cells may be attributable to improved supply of substrate used in energy production.

Erythrophagocytosis is not a prominent finding in fixed tissue sections of HS spleens. *In vitro* studies by Wright and associates⁶⁷ on phagocytosis of HS cells suggest that phagocytosis may nevertheless be another mechanism contributing to red cell destruction in this disease. That the red cells are in fact destroyed within the spleen, and not merely prepared for destruction elsewhere, is indicated by the relatively high concentration of bilirubin in blood from the splenic vein of HS patients.^{3,68}

TREATMENT

Splenectomy promptly, completely and permanently relieves anemia and jaundice in nearly all HS cases. In few other chronic diseases can the surgeon promise the patient so much. Dacie³ has reviewed the reported instances of recurrence of anemia after splenectomy and has concluded that all but two or three of the cases

were probably examples of acquired hemolytic anemia or non-spherocytic hemolytic anemia. Splenectomy can be recommended enthusiastically in all but the very mildest cases unless there are complications such as the presence of serious heart disease, senility or advanced malignancy. Even in relatively mild cases splenectomy may often be justified because of the threats of crises and development of gallstones. Decision regarding splenectomy is admittedly difficult, however, in a small portion of the cases encountered.

Case finding in hemolytic disorders, especially HS, has become a matter of importance to public health. Whenever a new HS patient is found, all available relatives should be examined so that other affected members of the family can be offered the benefits of splenectomy. Since the disease is so easily and effectively treated, we have never advised HS patients to limit the size of their families because of fear of passing on the HS gene. We have, however, advised that each child of an HS parent be examined within a few months after birth, or earlier if there are neonatal manifestations, to determine whether or not the disease has been inherited. If the child's blood is normal, the parents can be reassured and can be told, with slight theoretical reservation, that such a child will not transmit the HS gene to his offspring. If spherocytosis is found in the child's blood, splenectomy should be contemplated and performed at an elective date, preferably by the age of four or five years. Operation in infancy or early childhood may be justified if a crisis occurs or if the child's growth and development are not satisfactory.

During the operation the practice of squeezing the spleen to force red cells from this organ into the patient's circulation via the splenic vein should be discouraged. The red cells trapped in the spleen have much greater osmotic fragility than the red cells in the peripheral blood. It would therefore seem wiser to remove these cells with the spleen rather than to attempt to force them into the circulation. If gallstones have developed by the time splenectomy is performed, the surgeon may elect to remove the gallbladder along with the spleen. In many cases, however, it seems wiser to perform cholecystectomy at a later date, after the patient has recovered from splenectomy.

Transfusion is seldom necessary except in preparing the more severely affected patients for surgery or in treating the few patients who cannot or will not be splenectomized. Most HS

patients can be splenectomized without transfusion because the anemia is not often severe and because they are well adjusted to the anemia. Since the marrow is hyperplastic, the red cell count usually rises very rapidly after operation. As previously stated, transfused normal red cells usually survive normally in HS patients but may be destroyed rapidly in some cases during crises.

We have had no experience in this clinic with administration of cortisone or ACTH to HS patients but such therapy may be justified during crises, especially if there is evidence of an immune mechanism. In the absence of complications, there is no rationale for use of iron, liver extract, vitamin B₁₂, folic acid or other hematinic preparations in the treatment of this disease.

SUMMARY

Hereditary spherocytosis is probably inherited as a mendelian dominant with wide variations in expression of the gene. Although all reported HS patients are presumably heterozygotes, some are severely affected, others mildly affected, and still others (parents and siblings of propositi) may have a "carrier" state undetectable by physical examination or by currently used laboratory tests.

The principal hematologic findings in this disease are: increased thickness/diameter ratio and increased osmotic and mechanical fragility of the red cells, and rapid lysis of the red cells during sterile incubation at body temperature. Recent observations suggest that derangements in carbohydrate metabolism may prove to be largely responsible for some of these findings.

Probably because of their abnormal thickness, red cells of HS patients are readily trapped in the spleen and are thus quite likely deprived of adequate substrate for glycolysis. In this unfavorable situation the cells undergo lysis, most likely as a result of their metabolic "handicap." Once the splenic trap is removed the red cells have continuous access to substrate in the circulation and they have nearly normal survival time, despite persistence of abnormalities in shape, fragility and carbohydrate metabolism.

Further studies of this disease promise to add substantially to our understanding of the structure and metabolism of both normal and abnormal erythrocytes. Continued investigation of patients with hereditary spherocytosis should also help to elucidate the manner in which the spleen deals with a variety of abnormal red cells.

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Clinico-pathologic Conference

Sudden Onset of Anemia, Ecchymoses, Fever, Neurologic Signs and Stupor

STENOGRAPHIC reports, edited by Albert I. Mendeloff, M.D. and David E. Smith, M.D., are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient, B. P. (No. 229377) a sixty-one year old white unmarried woman, was admitted to the Barnes Hospital on November 19, 1953, complaining of exertional dyspnea and pallor. She had always enjoyed excellent health until two weeks before admission. She had worked as a telephone operator and in a shoe factory, had lived with her sister and had done light housework apart from the duties of her employment. She had never taken any medicines, nor consulted a physician prior to this illness. Her family history was non-contributory.

Two weeks prior to admission her sister had noted the patient's skin to have a sallow, pale tint, which progressively increased. At the same time the patient had begun to have episodes of light-headedness, especially when stooping over. On mild exertion she had noticed definite dyspnea and a fleeting substernal pain. Accompanying these symptoms was the spontaneous appearance of ecchymoses over her lower extremities. Three days before admission the patient stated that her appetite, previously good, had disappeared; on two occasions she had vomited dark material, which she believed not to be blood. The following day she was seen by her private physician, who told her that she had anemia; he administered 10 units of liver extract intramuscularly on that day, and repeated it on the day of admission. The patient also began to take oral liver extract two days and one day before admission. There had been no melena, hematuria, jaundice, abdominal pain or skin eruption.

Physical examination at the time of admission revealed the patient's temperature to be 38.4° C., pulse 98, respirations 18, and blood pressure

110/88. She was a well developed, cooperative woman who appeared chronically ill but in no distress. The skin was pale and yellowish; scattered small petechiae were noted over the thorax and both legs. There were several large ecchymoses over the thighs, hands, arms and legs. The conjunctivas were clear. A flame-shaped hemorrhage was seen in the left fundus near the optic disc, and a large hemorrhage below the disc. There was no lymphadenopathy. Examination of the chest and lungs was not remarkable. The heart was of normal size and exhibited a regular rhythm. A loud, high-pitched, blowing systolic murmur was heard in the third and fourth intercostal space at the left sternal border. The pulmonic second sound was increased in intensity, and the apical first sound duplicated. The liver was palpable 4 cm. below the right costal margin; the spleen was not felt. Pelvic and rectal examinations were within normal limits. Neurologic examination disclosed no abnormalities.

The laboratory examinations were as follows: red blood cell count, 1,700,000 per cu. mm.; hemoglobin, 6.3 gm. per 100 ml.; white blood cell count, 6,100; differential: segmented forms, 72 per cent; band forms, 6 per cent; myelocytes, 2 per cent; metamyelocytes, 1 per cent; lymphocytes, 10 per cent; monocytes, 9 per cent. Platelet count, 10,500 per cu. mm.; reticulocytes, 15 per cent. Four normoblasts were seen per 100 white blood cells. The red cells showed marked anisocytosis and poikilocytosis. Urinalysis: specific gravity, 1.012; pH, 7.0; albumin, one plus; sugar, negative; centrifuged sediment: 10 to 20 red blood cells and occasional white blood cell per high-power field; bile, negative; urobilinogen, positive in 1:8 dilution only. Stool:

guaiac negative. Cardiolipin test, negative. Blood chemistry: non-protein nitrogen, 16 mg. per cent; sugar, 125 mg. per cent; total serum protein, 5.8 gm. per cent; albumin, 3.9 gm. per cent; globulin, 1.9 gm. per cent; sodium, 141 mEq./L.; potassium, 3.4 mEq./L.; chloride, 105 mEq./L.; carbon dioxide combining power, 30.2 mM/L.; cephalin cholesterol flocculation, 3 plus; thymol turbidity, 1.5 units; total serum bilirubin, 1.6 mg. per cent; one minute fraction, 0.17 mg. per cent; prothrombin concentration, 80 per cent of normal; bleeding time, 35 minutes; clotting time, 5 minutes; clot retraction, none after 5 hours. Gastric analysis, no free acid after histamine stimulation. Urinary uropepsin, 0.12 log units per hour. L.E. test, negative. Coombs test: negative. Roentgenogram of the chest: minimal hypertrophic arthritis of the dorsal spine, otherwise negative. Electrocardiogram: borderline record showing vertical heart position.

On the second hospital day bone marrow aspiration revealed hypercellular marrow with marked hyperplasia of the nucleated red cell series and an occasional megaloblast. There was a slight increase in lymphocytes. Many phagocytic clasmacytocytes were present. The megakaryocytes were increased in number and were immature. This marrow was thought to be compatible with pernicious anemia following the institution of liver therapy but was not diagnostic. Reticulocyte count was 27 per cent. Beginning on the third hospital day the patient was treated with 200 μ g. of vitamin B₁₂ intramuscularly daily. On the fourth hospital day she suddenly became unresponsive; although this episode was transient, it was noted that the patient had aphasia. Neurologic examination at this time showed no abnormalities aside from aphasia. On the evening of the fourth day the patient had improved markedly, and although somewhat lethargic she was able to converse, responded to commands, and seemed well oriented. On the morning of the fifth hospital day the patient was noted again to have aphasia. This was thought to be a motor aphasia since the patient was unable to express herself although she did attempt to answer questions and seemed to understand requests. Physical examination at that time revealed that the deep tendon reflexes on the right were hyperactive. No pathologic toe sign could be elicited. Lumbar puncture was performed. Initial pressure was 140 mm.; final pressure, 90 mm.; cells,

0; protein, 52 mg. per cent; sugar, 76 mg. per cent; chloride, 122 mEq./L.

The patient was given 25 mg. of ACTH in 1,000 ml. 5 per cent glucose in water as a slow continuous intravenous drip. During this time her mental status varied. At times she was in deep coma, responding only to painful stimuli, and at other times she became more alert, combative, belligerent, and on occasion seemed to understand and respond to command. Following intravenous ACTH therapy the patient was given a platelet transfusion of 500 ml. fresh whole blood, collected in sequestrene, and 100 mg. of hydrocortisone intravenously. A repeat Coombs test was done and was questionably positive; a test for the presence of platelet agglutinins was negative. Large doses of cortisone (100 mg. every four hours via stomach tube) were administered. The course, however, was unfavorable. On the sixth hospital day the patient began to bleed from her mouth and nose. Her state of consciousness progressively decreased and on the sixth day neurologic examination revealed a right hemiparesis, including a right central facial paralysis and a positive Babinski sign on the right side. Blood counts consistently showed reticulocytosis and thrombocytopenia. On the eighth hospital day repeat blood counts were as follows: red blood count was 2.45 million; hemoglobin, 7.5 gm.; reticulocytes, 19.7 per cent, platelets, 4,000; differential count, as before. Bone marrow aspiration showed a cellular marrow. There was again an increase in megakaryocytes, most of them young forms. On the day of death the patient began to have muscular twitching and then had several generalized tonic and clonic convulsions. Following one of these episodes she suddenly expired, on November 28, 1953.

CLINICAL DISCUSSION

DR. VIRGIL SCOTT: The case under discussion presents us with the problem of the sudden onset in a middle-aged woman of a fulminating hematologic disorder. The rapid development of a profound anemia in the absence of obvious blood loss makes us consider immediately the possibility of intravascular hemolysis. From the protocol we see that the clinicians taking care of this patient went to considerable pains to define this and other mechanisms which might have accounted for her anemia. Dr. Chernoff, would you evaluate the laboratory data bearing on the hematologic problem?

Clinico-pathologic Conference

DR. AMOZ CHERNOFF: It seems to me that a hemolytic anemia is the best explanation for the laboratory data included in the protocol. In the absence of massive bleeding or a specific response to therapy such as to B_{12} or iron, the high reticulocyte count suggests most strongly a hemolytic process. Since neither the hematologic data nor the clinical history indicate sudden acute hemolysis, we must assume this patient was suffering from a more chronic hemolytic anemia. Corroborative evidence is given by the fact that the anemia was normocytic and normochromic, that the bilirubin was elevated with the indirect reacting fraction predominating, and the bone marrow showed erythrocytic hyperplasia. The absence of a positive Coombs test does not disturb me since even in acquired hemolytic anemia not more than 50 per cent will show a positive Coombs test.

DR. SCOTT: The physician who saw this woman in her home apparently believed she might have pernicious anemia, and it is interesting that after her arrival at Barnes Hospital liver and B_{12} therapy were continued. Dr. Moore, would you comment on the initial impression of your staff regarding the diagnosis, and at what point this diagnosis was abandoned?

DR. CARL V. MOORE: In retrospect, it may seem peculiar that a diagnosis of pernicious anemia was seriously considered when this patient was first seen, particularly since her red blood cells were not macrocytic. On the other hand, the following changes were compatible with pernicious anemia: marked anisocytosis and poikilocytosis of the erythrocytes, leukopenia, thrombocytopenia, macrometamyelocytes, histamine refractory achlorhydria and reticulocytosis plus erythroid hyperplasia, without maturation arrest, four days after therapy with liver extract. The reticulocytosis of 27 per cent on the fourth day of therapy was a little extreme in a patient whose red blood cell count was 1.7 million. The thrombocytopenia was more profound than one sees in pernicious anemia except on the rarest of occasions; furthermore, the platelets should have begun to increase concurrently with the reticulocytosis if the latter had been induced by liver therapy. Within a few days, it became obvious that a diagnosis of pernicious anemia was incorrect: there was no clinical improvement, no change in the reticulocyte level, no rise in the red blood cell count and no increase in platelets.

DR. SCOTT: Dr. Loeb, you suggested another

diagnosis in this case, a very rare hematologic disorder, soon after the patient was admitted. Would you care to tell us the reasoning which led you to make this diagnosis?

DR. VIRGIL LOEB, JR.: As has been pointed out in the previous discussion it became obvious within a few days that a diagnosis of pernicious anemia was not tenable. After we had had a chance to observe the patient for a while and accumulate some laboratory data a more careful analysis of the problem resolved itself along the following points. We were dealing with a woman who developed a rather explosive hemorrhagic disease several weeks prior to admission. Complicating the spontaneous bleeding was the fairly rapid onset of nausea and severe vomiting, associated also with dyspnea and dizziness. Marked pallor and hepatomegaly were noted in addition to the petechiae and ecchymoses. Under our observation she ran a febrile course which was also characterized by transitory restlessness, confusion and stupor; from time to time convulsions and facial weakness were noted. Laboratory data indicated a severe thrombocytopenic purpura and hemolytic anemia with nucleated red blood cells in the peripheral blood. Attempts to demonstrate auto-immune antibodies with the direct and indirect Coombs technic were negative and neither platelet agglutinins nor L.E. cells could be detected. For this reason a diagnosis of thrombotic thrombocytopenic purpura was considered. The symptomatologic triad of thrombocytopenic purpura, hemolytic anemia and transitory, bizarre neurologic signs was certainly present in this case and the laboratory data were compatible with such a diagnosis.

DR. SCOTT: I have asked Mr. Daniel Nathans, of the senior class, who also suggested the diagnosis of thrombotic thrombocytopenic purpura in this patient, to review for us the generally accepted pathologic and clinical features of this unusual disease.

MR. DANIEL NATHANS: The pathology as well as most of the clinical features of thrombotic thrombocytopenic purpura was first described by Moschcowitz in 1925,¹ and, according to a recent article,² approximately forty cases have

¹ MOSCHCOWITZ, E. An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries; an undescribed disease. *Arch. Int. Med.*, 36: 89-93, 1925.

² COOPER, T., STICKNEY, J. M., PEASE, G. L. and BENNETT, W. A. Thrombotic thrombocytopenic purpura. *Am. J. Med.*, 13: 374-383, 1952.

since been added to the literature. The characteristic and most striking pathologic change consists of myriads of hyaline thrombi in small arteries and arterioles in almost every tissue of the body. The brain is usually very prominently involved, as might be expected from the clinical signs referable to the central nervous system, and the myocardium, spleen and bone marrow are other sites of fairly constant involvement.

It was suggested early in the history of this syndrome that the characteristic thrombi were composed of platelets and most of the following authors have agreed. It is, nevertheless, to be admitted that the evidence is not conclusive. No morphologic forms are recognizable within the rather homogeneous thrombi of either platelets or other formed elements such as erythrocytes, leukocytes or fibrin. Special staining procedures such as the periodic acid-Schiff reaction and phosphotungstic acid-hematoxylin technic demonstrate that the thrombi do not stain at all like ordinary fibrin or fibrous tissue but do show the types of staining reactions that result from staining a mass of separated platelets. These reactions are characteristic but not specific for platelets, however, and there is no specific stain for these elements that would conclusively demonstrate the nature of the thrombi.

It has more recently been suggested that the primary site of the pathologic alteration might be within the vascular wall rather than the elements of the blood. This is, of course, supported by the generally accepted concept that thrombocytopenia without vascular damage results in no significant bleeding. Orbison³ has described focal amorphous eosinophilic lesions that appear to be entirely within the walls of vessels with patent lumens, and interprets them as early stages of the occlusive lesion. He has also shown by means of serial reconstruction that the involved vessels are segmentally dilated to form small fusiform aneurysms at the sites of the lesions. The interpretation of a primary lesion within the wall of the blood vessel has, of course, led to the suggestion that this may be another "collagen disease."

The other significant alterations in the tissues are of a secondary nature and do not contribute much to clarification of the pathogenesis. In the bone marrow there is erythroid hyperplasia and increased numbers of immature megakaryo-

³ ORBISON, J. L. Morphology of thrombotic thrombocytopenic purpura with demonstration of aneurysms. *Am. J. Path.*, 28: 129-144, 1952.

cytes, as may be seen in other purpuric diseases. The reticuloendothelial tissues show evidence of the hemolytic character of the anemia in the form of diffuse hemosiderosis. Small extravasations of blood are widely disseminated, but it is noteworthy that frank infarction of tissue is uncommon.

Laboratory examinations have fairly clearly indicated that the anemia in this syndrome is hemolytic in character without alteration in the fragility of the erythrocytes. Reported serologic and immunologic tests have not shown significant positive values, e.g. tests for "L.E. cells" and the Coombs test have been negative, but apparently there has never been the opportunity to explore all presently available procedures.

DR. SCOTT: Because there is some reason to attribute the reaction in the vessel walls, if they truly exist, to a form of collagen disturbance, and since the Shwartzman reaction has been specifically cited as a possible cause for such a necrotic lesion, it might be well for us to have Dr. BUKANTZ review the present ideas of the nature of this reaction.

DR. SAMUEL BUKANTZ: After a series of very extensive observations, Shwartzman published a monograph on this subject which he was always very careful to entitle, "Phenomenon of Local Tissue Reactivity."⁴ This evasive terminology was deliberate on Shwartzman's part, since he always stressed that the phenomenon was not one due to hypersensitivity in the same sense as the Arthus reaction. The phenomenon that Shwartzman studied consists of an intense hemorrhagic and necrotic skin reaction in rabbits produced under specific circumstances. Filtrates of young agar culture washings of *Eberthella typhosa*, or a variety of other organisms, are injected intradermally (preparatory injection). On injection intravenously, eight to thirty-two hours later, a small amount of the filtrate (provocative injection), will provoke the phenomenon. The initially injected skin site quickly undergoes hemorrhagic necrosis and the reaction reaches its height about five hours after the intravenous injection.

The essential conditions for this reaction as studied in the experimental animal are: (1) the provocative injection must be given intravascularly; (2) the provocative injection must follow within a limited interval of time after the preparatory injection; and (3) there is no

⁴ SHWARTZMAN, G. Phenomenon of Local Tissue Reactivity. New York, 1937. Paul B. Hoeber, Inc.

antigenic specificity in the preparatory and provocative injection; non-antigenic substances such as agar or stock solutions will also serve as provocative agents. These characteristics distinguish the phenomenon from pathologically similar reactions of hypersensitivity and induced Shwartzman to use a descriptive title to label it as such.

The most energetic students of this phenomenon have always been deeply interested in the possibility that a number of unexplained clinical events might, in the human, be the result of a similar mechanism. Thus Sanarelli,⁵ in 1924, while working with the same phenomenon, observed striking intestinal reactions in rabbits receiving sublethal doses of cholera vibrio followed by small numbers of colon bacilli intravenously. These animals died unexpectedly and their intestinal mucosa revealed areas of necrosis. Sanarelli believed that many of the acute abdominal crises in humans might have a similar basis. Shwartzman, himself was for some years greatly interested in the possibility that some of the toxic manifestations of bacterial infections might result from similar phenomena—and could possibly be ameliorated by the use of antisera of demonstrated effectiveness in preventing the phenomenon in the experimental animal.

Apitz,⁶ in 1935, observed diffuse vascular and degenerative changes in the kidneys, lungs, liver and heart of animals exhibiting a generalized reaction following successive injections of bacterial filtrates intravenously at twenty-four-hour intervals. Such observations as these have been partly responsible for the frequent attempt to apply the phenomenon to explain certain disease states in the human, among which, of course, is the thrombotic thrombocytopenic purpura under consideration today.

The several publications by Thomas, Stetson and Good,⁷ since 1949, have dealt with an analysis of the cellular biochemical alterations associated with the provocation of the Shwartzman phenomenon. They have observed, at the

⁵ SANARELLI, G. Experimental cholera. *Ann. de l'Inst. Pasteur*, 38: 11, 1924.

⁶ APITZ, K. Study of generalized Shwartzman phenomenon. *J. Immunol.*, 29: 255, 1935.

⁷ (a) THOMAS, L. and STETSON, C. A. Studies on mechanism of Shwartzman phenomenon. *J. Exper. Med.*, 89: 461, 1949; (b) STETSON, C. A. and GOOD, R. A. Studies on mechanism of Shwartzman phenomenon; evidence for participation of polymorphonuclear leucocytes in phenomenon. *J. Exper. Med.*, 93: 49, 1951.

prepared site, an increase in aerobic glycolysis with lactic acid formation, for which the accumulation of polymorphonuclear leukocytes is essential. The accompanying injury of the endothelium of the vessels upon injection of the provocative factor leads to platelet and granulocyte thrombi which interrupts blood flow resulting in local necrosis.

Such pathologic changes in the experimental animal are not greatly different from some of the changes noted in thrombotic thrombocytopenic purpura and certainly make for speculation concerning the operation of Shwartzman phenomenon-like biochemical alterations as the pathogenetic mechanism of the disease.

DR. SCOTT: Treatment of this fulminating disease was carried out principally by means of transfusions and steroid hormones. Insofar as I am able to learn, the only other method of treatment sometimes employed is splenectomy, but I am under the impression that there is no reliable therapeutic measure which will stop the progress of this very severe disorder. Would you comment on the rationale of therapy given to this patient, Dr. Loeb?

DR. LOEB: The question of treatment in this patient posed many problems since proof of the diagnosis was lacking and we had no personal experience upon which to base our decisions. A bone marrow biopsy was contemplated in an attempt to demonstrate the pathologic lesions of this syndrome, but this was not done because of the fear of severe hemorrhage secondary to the thrombocytopenia. Our decision not to do a splenectomy was based on two lines of reasoning. In the first place, our experience in the past with idiopathic thrombocytopenic purpura has indicated that it is those patients in whom platelet agglutinins can be demonstrated that seem to be benefited by splenectomy, whereas those without demonstrable antibodies do not respond as well. In the second place a review of the previously reported cases of thrombotic thrombocytopenic purpura indicated that seven of nine patients with this disease failed to respond to splenectomy. In the other two cases the rise in platelet count was only temporary. Blood transfusions were given to this patient, but no concerted attempt to transfuse platelets was carried out. One patient with this syndrome has been reported, in whom it was found that platelets were removed rapidly from the circulation.⁸ ACTH and cortisone were given to this

⁸ ADELSON, E. and STEPANINI, M. Studies on platelets.

patient on a relatively empirical basis, because we have seen highly beneficial results from these drugs in many cases of acquired hemolytic anemia and thrombocytopenic purpura. One fairly constant effect of these hormones has been to decrease capillary permeability, even if there is little effect upon the platelet level; one of our main reasons for continuing this therapy was to prevent further hemorrhage. In retrospect, it is obvious that the treatment which we gave her was ineffective for whatever disease she had; perhaps we were in error in not recommending immediate splenectomy at the time the diagnosis was entertained.

DR. SCOTT: In summary, there seems little doubt that this patient was suffering from thrombotic thrombocytopenic purpura, and it would be most surprising if the pathologists will be unable to confirm this diagnosis.

PATHOLOGIC DISCUSSION

DR. RICHARD L. SWARM: The gross findings in this case were largely limited to petechiae and ecchymoses which were present in the skin, the pleura, the heart and in sections of the brain, in which there were numerous very small and somewhat indefinite lesions. There was a very small amount of serous fluid in the pleural cavities. The liver and spleen were normal in size and the liver appeared to be slightly icteric without a recognizable generalized icterus of the other viscera. The femoral marrow was found to be pale and fatty. There were some gastric contents in the trachea and bronchi which we associated with the terminal episode.

DR. DAVID E. SMITH: The first microscopic illustration (Fig. 1) is of a section of the myocardium in which the essential lesion of this disease is clearly shown. The involved vessel is an arteriole and probably a branch of the patent vessel in the lower right corner. By searching through a number of microscopic sections the continuity between uninvolved and involved arterioles can be observed, as has been demonstrated by others by means of serial reconstruction. The lumen of the involved vessel in this illustration is completely occluded by a rather amorphous mass of material. This material is positive to the periodic acid-Schiff stain, negative to the phosphotungstic acid stain, and negative to the usual stains for fibrin

and collagen. These tests are, of course, only presumptive, but they are consistent with the suggestion that the material is derived from platelets and not from fibrin and fibrous tissue. Morphologic identification of platelets in these masses, however, is not possible in either this case or those reported by others. The vessel wall shows hyperplasia of the endothelium, and in this particular vessel that is the only apparent alteration. It is significant that the wall of the adjacent, probably proximal vessel, is essentially unremarkable. Figure 2 is a higher magnification of the lesions in much smaller vessels. Both of the vessels are filled by masses of thrombotic material. The smaller mass is probably more indicative of the pathogenesis of the lesions in that it is discretely contained within the former outlines of the vessel even though it is covered by endothelium. This suggests that this lesion actually consists of something that has been laid down on the endothelium and has been covered over by it.

The argument as to whether these are lesions of the vascular wall that rupture through the endothelium, or whether they are something like a thrombus attached to the endothelium and later incorporated into the wall is impossible to settle by present morphologic methods. The intact layer of endothelium over the masses is not evidence against their being primarily thrombi, for experimental work⁹ on emboli and blood clots has shown that small emboli which become attached to the wall of the vessel are covered over by endothelium very rapidly and are capable of initiating a destructive lesion in the underlying wall. Just exactly how that destruction is accomplished is hard to understand, but the experiments indicate quite clearly that in animals in which emboli become attached to the vascular walls, the wall of the vessel often undergoes disruption. Those who would maintain that the primary lesion of this disease is in the vascular wall can demonstrate little more than endothelial hyperplasia in vessels that are not involved by the thrombi, whereas the presence of thrombi without appreciable destruction of the vessel is easily demonstrated in this disease as well as in experiments with thrombotic emboli.

Figure 3 is a lower magnification of the edge

⁹ WARTMAN, W. B., JENNINGS, R. B. and HUDSON, B. Experimental arterial disease, the reaction of the pulmonary artery to minute emboli of blood clot. *Circulation*, 4: 747-755, 1951.

vi. Demonstration and characterization of a heterologous (Forsmann) platelet agglutinin. *Blood*, 7: 700, 1952.

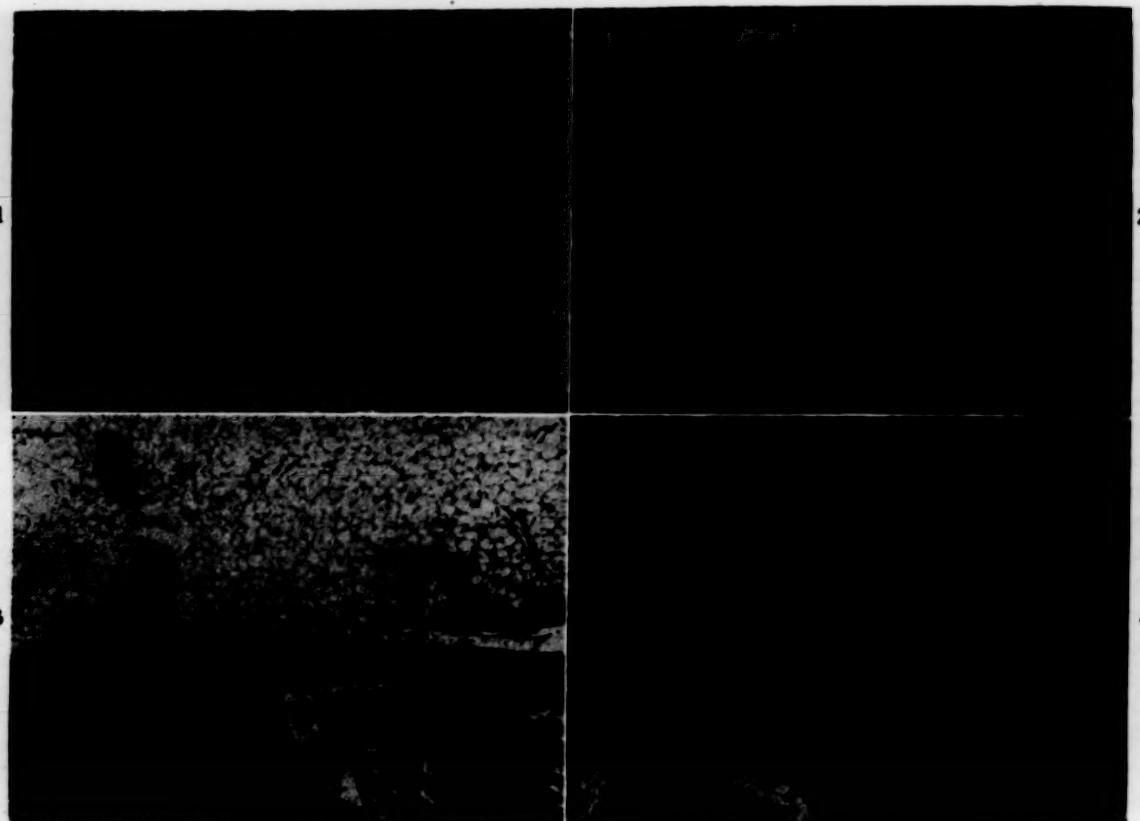


FIG. 1. Myocardium showing a vessel occluded and distended by a mass that is homogeneous and amorphous. These masses do not stain in the manner of fibrin but do have histochemical reactions that are consistent with, although not conclusive of, a derivation from platelets.

FIG. 2. Two small vessels in the myocardium with included thrombi. A covering of these thrombi by endothelium is shown and in the smaller vessel the complete containment of the lesion within a normal vascular wall can be seen.

FIG. 3. The epicardium to show the widespread presence of the thrombi in vessels throughout this tissue.

FIG. 4. An afferent arteriole in the glomerulus containing a thrombus but no lesions in the glomerulus. Partial occlusion of the afferent vessel is the only apparent explanation for absence of lesions in the glomerulus, for this site allows the development of no collateral to its peripheral structures.

of a section of the heart to show the extent of involvement of the vessels by the thrombotic process. In many areas almost every vessel is involved, and in this illustration at least six vessels in the epicardium, and a large vessel in the myocardium, show the lesions. The absence of reaction in the surrounding tissue is noteworthy and characteristic of almost all involved tissues in this disease. Figure 4 is from a section of a renal glomerulus showing a thrombus in an afferent arteriole. Again it is striking that the glomerulus is relatively unaltered. It has been suggested that these thrombi are essentially mural thrombi that do not completely occlude the lumen, thus explaining the absence of infarction. The gastric mucosa, adrenal, spleen and almost every other

tissue examined in this case are involved by similar thrombi.

In the brain (Fig. 5) a large number of vessels are involved, principally in the lower part of the cortex. Endothelial hyperplasia is especially prominent in this tissue in vessels that are not involved by the thrombi. One would have to do serial reconstructions to decide the exact relation of these vessels to the thrombi, but I should guess that they are distal. Certainly an endothelial thickening or hyperplasia of empty distal blood vessels is often seen after occlusion of major arteries in the brain and is apparently a reaction of the lining of those vessels to hypoxia. Figure 6 shows a small rather well delimited area in the cerebral cortex in which there is rarefaction of stroma and



FIG. 5. Brain showing many lesions particularly in the deeper part of the cortex. Many vessels not involved by thrombi also show prominent endothelial hyperplasia.

FIG. 6. A small area in the lower part of the cerebral cortex showing rarefaction of stroma and shrinkage and dark staining of neurons indicative of destruction of nerve cells in this case.

FIG. 7. The bone marrow showing many small dark erythroblasts indicative of erythroid hyperplasia. Numerous megakaryocytes contain decreased numbers of nuclei and an immature appearance. The arteriole in the section is occluded by a typical thrombus.

FIG. 8. The spleen showing extramedullary hemopoiesis. Diffuse hemosiderosis is also a prominent feature of this tissue.

shrinkage and pyknosis of the neurons, in contrast to the appearance of the cells in the adjacent normal cortex. This indicates that there is actual neuronal damage in the brain as might well be expected from the prominent clinical signs relative to the central nervous system, and in contrast to the generally innocuous presence of the thrombi in other tissues. In a series of ten sections taken throughout many regions in this brain multiple thrombi were found in eight.

In reference to the anemic component of this disease, the bone marrow (Fig. 7) is the seat of erythroid hyperplasia as indicated by the collections of numerous small, dark erythroblasts. The fairly numerous megakaryocytes, as was reported in the clinical examination, have decreased numbers of nuclei and an immature appearance that is often seen in purpura. A

small vessel on the right side of the picture is occluded by a typical thrombus. It is interesting that Cooper and co-workers have described a case in which they were able to make an antemortem diagnosis of this disease by recognition of the thrombi in a section of an aspirated bone marrow biopsy, although they did not find the thrombi in film preparations. The spleen (Fig. 8) also shows the effect of hemolysis and anemia by the large masses of extramedullary hemopoiesis and widely disseminated hemosiderosis. Thrombi are also present in this organ; in some cases the diagnosis has been made by histologic examination of the spleen following splenectomy. It has been suggested that skin or muscle biopsy might be helpful in making a diagnosis before death, but in the present case as well as in most of the reported

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cases random sections of these tissues at autopsy have failed to demonstrate the typical lesions.

In summary, this case showed all of the anatomic manifestations of thrombotic thrombocytopenic purpura: multiple small thrombi throughout small arteries and capillaries in many tissues, with relatively scanty effect on the surrounding tissues, and evidence in the bone marrow and spleen of increased destruction of erythrocytes. Purpura is apparently a rather inappropriate terminology for this disease, as these patients do not show all the hemorrhagic manifestations of other forms of purpura. Gross ecchymoses and large hemorrhages are not the rule, although petechiae and small ecchymoses are widespread. The scanty evidence of reaction in the tissues surrounding

the involved vessels is a remarkable phenomenon, and in this case damage is clearly present only in small foci in the brain.

Final Anatomic Diagnoses: Thrombotic thrombocytopenic purpura, with thrombi in the small arteries and arterioles of the brain, heart, adrenals, kidneys, liver, spleen, stomach, pancreas, bone marrow and salivary gland; petechiae and ecchymoses of the skin and mucous membranes, brain, epicardium, myocardium, endocardium, and mucosa of the stomach, renal pelvis and urinary bladder, slight.

Acknowledgment: Illustrations were made by the Department of Illustrations, Washington University School of Medicine.

Case Report

Pluriglandular Insufficiency Simulating Panhypopituitarism*

Report of Two Cases

J. J. RUPP, M.D. and K. E. PASCHKIS, M.D.

Philadelphia, Pennsylvania

PLURIGLANDULAR insufficiency manifested by hypothyroidism, hypoadrenalinism and hypogonadism usually is the result of severe insufficiency of the adenohypophysis.^{1,2} The multiglandular insufficiency develops as a result of the absence of the pituitary trophic hormones which are necessary for maintenance of the normal size and the normal function of the thyroid gland, the adrenal glands and the gonads. The possibility that pluriglandular insufficiency simulating hypopituitarism could develop as a result of multiple glandular failure secondary to multiple glandular sclerosis was suggested by Falta.³ However, the cases of multiple endocrine gland sclerosis reported by Falta, as well as the examples of this syndrome he collected from the literature, had pituitary disease⁴ and might well have been examples of panhypopituitarism.

We are aware of three reports of cases of pluriglandular insufficiency simulating panhypopituitarism.⁵⁻⁷ The patients who were the subjects of these reports had diabetes mellitus as well as hypothyroidism and hypoadrenalinism. In each case the pituitary gland was normal by gross and microscopic examination. In one case the adrenal cortices were absent,⁶ in the second they were fibrotic,⁵ and in the third they were normal by gross and histologic examination.⁷ In all three cases the thyroid gland was atrophic and fibrotic. The testes were normal in the two male patients;⁵⁻⁷ the ovaries of the female patient⁶ were grossly normal; histologic examination revealed follicle cysts and the absence of corpora lutea.

Inasmuch as cases of primary endocrine gland failure simulating panhypopituitarism are rare, it was deemed of interest to report the studies of two patients who had hypoadrenalinism, hypothyroidism and hypogonadism with normal pituitary function.

CASE REPORTS

CASE 1. In 1947 E. D., a thirty-four year old white housewife, was admitted to the medical service of the Jefferson Medical College Hospital. She had been in good health until two years prior to hospitalization, at which time she noticed increasing pigmentation of the skin. Six months prior to admission she first noticed weakness, easy fatiguability and anorexia. During this six-month period she lost 30 pounds, the weakness increased and hypotension developed. Six weeks prior to hospitalization abdominal discomfort, nausea and vomiting first occurred, and recurred with such frequency that hospitalization was advised.

There was no history of, or exposure to, tuberculosis. In 1937 the patient was delivered of a normal child after an uneventful pregnancy. Her menses returned but were irregular until six months prior to hospitalization, when they ceased.

Physical examination at the time of admission revealed increased pigmentation of the skin, creases of the hands, axillary folds and buccal mucous membranes. The body hair was normal in amount and distribution. Pelvic examination was negative, although the uterus was smaller than that expected during the child-bearing age.

* From the Division of Endocrine and Cancer Research, and the Department of Medicine, Jefferson Medical College, Philadelphia, Pa.

The pulse rate varied between 70 and 90 beats per minute, the blood pressure between 80/50 and 110/80.

The results of the laboratory studies were as follows: Hemoglobin was 70 per cent. The red blood cell count was 3,700,000. The white blood cell count was 5,000 with a normal differential count. Frequent routine urine analysis yielded normal results. Serologic tests for syphilis were negative. Serum calcium was 11 mg. per 100 ml. Cholesterol was 210 mg. per 100 ml. The basal metabolic rate was minus four. The Cutler test⁸ was positive; on the third day of the test the urine chloride excretion was 443 mg. per 100 ml. During the test the patient experienced an increase in weakness and nausea, and her blood pressure fell to lower levels. The urinary 17-ketosteroids⁹ were 1.2 mg. equivalents of androsterone. The twenty-four-hour urinary excretion of gonadotrophins was 182 units as determined by the mouse uterine weight method. The insulin tolerance test¹⁰ revealed "hypoglycemic irresponsiveness." The blood sugar in mg. per 100 ml. at the stated times following the intravenous injection of 0.1 unit of insulin per kilogram was: 0 min. 70, 20 min. 25, 30 min. 25, 45 min. 32, 60 min. 20, 90 min. 28, 120 min. 33. The electrocardiogram showed low voltage in all leads. Roentgenogram of the chest was negative except for a decrease in the heart size from that expected for her height and weight. Roentgenogram of the abdomen did not reveal any calcifications. The roentgenogram of the skull was negative.

The diagnosis of Addison's disease was made and the patient was treated with desoxycorticosterone acetate and salt. She gained a few pounds, had a return of appetite and strength, and the gastrointestinal symptoms disappeared.

During the following two and a half years the patient was seen frequently and was well. The 17-ketosteroid excretion remained low, and the urinary gonadotrophin excretion high. The basal metabolic rate varied between plus ten and minus six. The serum cholesterol was 208 mg. per 100 ml. on one occasion, and 242 mg. per 100 ml. on another. The sensitivity to insulin ("hypoglycemia irresponsiveness") and the positive response to the Cutler test were unchanged. An electroencephalogram showed an abnormally slow rate without dysrhythmia. During this period a tracer study with I-131 was performed in the course of a survey of thyroid function in Addison's disease; at that time the patient did

not present any clinical manifestations suggestive of a disturbance in thyroid function. The uptake twenty-four hours after the ingestion of the tracer dose was 20 per cent.

Three and a half years after the patient's initial admission puffiness of the face, dryness of the skin, cold sensitivity, huskiness of the voice and lethargy developed. The 17-ketosteroid excretion remained less than 2 mg. equivalents of androsterone per twenty-four hours. The gonadotrophin excretion was greater than 196 mouse uterine weight units per twenty-four hours. The basal metabolic rate was minus twenty-two, the cholesterol was 350 mg. per 100 ml. The uptake of I-131 by the thyroid gland was now 13 per cent at twenty-four hours. Thyroid-stimulating hormone (TSH) assayed in the serum by the stasis tadpole method¹¹ was present in normal amounts.* Desiccated thyroid, 0.06 gm., was prescribed in addition to the desoxycorticosterone acetate and salt.

Four and a half years after her initial admission the patient was hospitalized for the ninth and last time for a routine check-up. She was feeling well and her weight was maintained. She had not had any change in the hyperpigmentation of the skin or of the body hair. The signs of hypothyroidism had disappeared. She had no vaginal bleeding for five years. The blood pressure was maintained at normal levels. The sensitivity to insulin and sodium restriction were not altered. The urinary excretion of gonadotrophins remained high and that of the 17-ketosteroids low. The basal metabolic rate was minus ten.

Two weeks after discharge abdominal pain suddenly developed in the patient. She was admitted to another hospital where she died shortly after admission. Autopsy was not performed.

CASE II. W. C., a fifty-seven year old white night watchman, had been in good health until 1947, one year prior to admission to the medical service of Jefferson Medical College Hospital. At that time he experienced dyspnea and easy fatigability. During the following year these symptoms increased; anorexia, with a weight loss of 35 pounds, and weakness developed. Two weeks prior to admission abdominal discomfort occurred. The patient was admitted to the hospital one year after the onset of symptoms.

Physical examination at the time of admission

* We are indebted to Dr. S. A. D'Angelo for performing this assay.

revealed a cyanotic hue of the lips and nail beds. Examination of the chest was negative except for bronchovesicular breath sounds with an occasional sonorous rale. Examination of the abdomen was negative. The body hair was normal in amount and distribution. The testes were somewhat small. The attending staff was not impressed with the degree of pigmentation of the skin; however, the family physician had noted that the skin pigmentation had increased during the three months prior to hospitalization. The pulse rate varied between 80 and 90 per minute, the blood pressure between 110/70 and 90/50.

Results of the laboratory tests were as follows: Hemoglobin was 90 per cent, red blood cell count was 4,800,000, white blood cell count was 8,100 with a normal differential count. The serologic tests for syphilis were negative. Routine urine analyses were performed frequently with normal results. The plasma proteins were 6.0 gm. per 100 ml., the albumin/globulin ratio was 2.5. Results of gastric analysis, tests of hepatic function and of renal function were normal. The results of the intravenous glucose tolerance test were (blood sugar in mg. per 100 ml. at stated times): 0 hr. 91, one-half hour 156, one hr. 190, two hr. 62, three hr. 64, four hr. 84, five hr. 57, six hr. 72. The insulin tolerance test revealed moderate "hypoglycemia irresponsiveness" (the blood sugar at the stated intervals following the intravenous injection of 0.1 unit of insulin per kg. body weight was): 0 min. 79, 20 min. 50, 30 min. 34, 45 min. 41, 60 min. 49, 90 min. 49, 120 min. 53. The salt deprivation test of Cutler was performed on two occasions and was positive each time; the urine chlorides were 230 mg. per 100 ml. and 354 mg. per 100 ml., respectively. The urinary excretion of 17-ketosteroids was 3.5 mg. and 3.8 mg. equivalent of androsterone per twenty-four hours. The urinary excretion of gonadotrophins was 66 mouse uterine weight units per twenty-four hours. Roentgenogram of the chest showed bilateral pulmonary emphysema; the heart size was within normal limits. The roentgenographic examination of the skull and of the gastrointestinal tract were normal. The electrocardiogram was within normal limits. The diagnosis of Addison's disease was made.

The patient insisted upon leaving the hospital before treatment of the adrenal insufficiency could be started. He was instructed to use two mg. of desoxycorticosterone acetate by the

buccal route, and 6 gm. of sodium chloride daily. He failed to report to the clinic for the next two years.

Two years later the patient was readmitted with the complaints of dyspnea, orthopnea and edema. He had taken 4 gm. of sodium chloride daily, and had been relatively well until six weeks prior to this hospitalization when an increase in dyspnea and in weakness, as well as recurrence of abdominal pain accompanied by nausea and vomiting developed. For three weeks prior to admission the patient had been treated by a physician with 5 mg. of desoxycorticosterone acetate by intramuscular injection every other day. During the two weeks prior to admission the weight had increased about 30 pounds, and the edema, orthopnea and dyspnea increased. At the time of admission the pulse rate was 100 per minute, the blood pressure was 134/70. The neck veins were distended; tachypnea, dyspnea and orthopnea were present. Examination of the chest revealed the signs of pulmonary edema. The liver was enlarged and tender. There was edema of the lower extremities and scrotum. At this time the hyperpigmentation of the hands was definite. Roentgenogram of the chest showed the heart size to be 30 per cent above normal. The electrocardiogram showed changes suggestive of hypopotassemia (flattening of the T waves with a prolongation of the Q-T interval). Bedrest, salt restriction and the use of mercurial diuretics resulted in a weight loss of 30 pounds in an eight-day period. The signs and symptoms of congestive heart failure disappeared. The results of the laboratory studies performed after recovery from the congestive failure are as follows: hemoglobin was 97 per cent, red blood cell count was 4,900,000; the white blood cell count was 7,600. The serum calcium was 10.8 mg. per 100 ml., phosphorus 3.5 mg. per 100 ml. The plasma proteins were 8.1 gm. per 100 ml., the albumin/globulin ratio was 1.85. The results of the liver function and kidney function tests were normal. The total eosinophil count prior to the intramuscular injection of 25 units of ACTH was 438 per cu. mm., four hours later the eosinophil count was 386 per cu. mm. The urinary excretion of 17-ketosteroids was determined in three different twenty-four-hour urine specimens and varied between 3.2 mg. and 4.0 mg. equivalent of androsterone. The twenty-four-hour urinary excretion of gonadotrophins was determined four times, and was greater than 196 mouse uterine weight

units on each assay. The salt deprivation test of Cutler (performed two weeks after the last injection of a mercurial diuretic) was positive; the urine chlorides were 253 mg. per 100 ml. The sensitivity to insulin had increased, the blood sugar (mg. per 100 ml. at the stated times after the intravenous injection of 0.1 unit of insulin per kg.) was: 0 min. 60, 20 min. 11, 30 min. 30, 45 min. 20, 60 min. 20, 90 min. 20, 120 min. 14. The basal metabolic rate was now minus twenty, the cholesterol was 310 mg. per 100 ml. The electrocardiogram showed right axis deviation without the previously described changes suggestive of hypopotassemia. Roentgenogram of the chest showed that the heart size had decreased to normal, but not to subnormal size. The patient was treated with 2 mg. desoxycorticosterone acetate by intramuscular injection, and with 4 gm. of sodium chloride daily. Within eight days he had gained 8 pounds, and again had signs and symptoms of congestive heart failure. These symptoms disappeared when the drug was discontinued. The patient again left the hospital against advice. He returned to the Outpatient Department where he was treated with methyltestosterone, 50 mg. daily, and 4 gm. of sodium chloride daily. For the next three months he was well, the blood pressure was within normal limits, slight weight gain occurred, there was no increase in dyspnea or any signs of fluid retention. For the following three months he was treated at home with 1 cc. of lipoadrenal extract as well as with methyltestosterone. Six months after his second hospitalization he was readmitted in coma, with signs of congestive heart failure and auricular fibrillation; he died shortly after admission.

The findings at autopsy were: * Fibrosis and emphysema of the lungs with pleural effusion and pulmonary congestion. The heart weighed 400 gm., the right ventricle was hypertrophied, measuring 8 mm. in thickness. The endocardium, valve leaflets and the coronary vessels were normal. The liver was small, weighing only 960 gm. The thyroid gland was smaller than normal, and histologic examination showed very little increase in fibrous tissue and no lymphocytic infiltration. The adrenal glands were small; histologic examination showed that the cortex was thin but the architecture was normal. The pancreas was normal on gross and microscopic examination. The testes were atrophic;

* Autopsy performed by Dr. M. Leiber, Department of Pathology, Jefferson Medical College.

histologic examination showed severe fibrosis with a decrease in the tubules and in the Leydig cells. Gross and microscopic examination of the pituitary gland were normal.

COMMENTS

The hypoadrenalinism, hypothyroidism and hypogonadism which these two patients exhibited was evidently the result of failure of the respective glands (primary pluriglandular insufficiency) rather than the result of primary pituitary insufficiency. The postmortem examination revealed a normal pituitary gland in Case II, and in each case the clinical manifestations as well as the results of the various tests performed indicated the presence of a functioning pituitary gland. The cause for hospitalization of these patients was hypoadrenalinism. The diagnosis of adrenal insufficiency was suggested by the clinical manifestations (hyperpigmentation, weakness, anorexia, weight loss) and was confirmed by the tests performed. Both patients showed a decreased urinary excretion of 17-ketosteroids, increased sensitivity to insulin ("hypoglycemia irresponsiveness"), and had a positive response to the salt deprivation test of Cutler. The hyperpigmentation which initially was present in Case I and later developed in Case II would indicate that the adrenal failure was primary rather than secondary to pituitary failure. Skin pigmentation does not increase when adrenal insufficiency is secondary to pituitary failure.^{1,2} Primary adrenal failure without pigmentation is rare.¹²⁻¹⁴ When Addison's disease occurs without hyperpigmentation the adrenal failure is probably associated with pituitary changes.¹⁵

At the initial hospital admission both patients showed hypogonadism which could not have been the result of pituitary inadequacy inasmuch as the urinary excretion of gonadotropins of both patients was at a high level. Titers of urinary gonadotropins such as was excreted by these patients occur in patients with primary gonadal failure, and result from overproduction of these hormones by a pituitary gland whose function in respect to gonadotrophin production is no longer regulated by the sex steroids. The gonadal failure associated with pituitary failure is the result of the lack of gonadotrophins, and these hormones no longer are found in the urine. The histologic changes found in the testes of Case II could have developed either as a manifestation of pituitary failure or of primary

testicular failure; atrophy of the testes, sclerosis of tubules, and a decreased number of Leydig cells occur also in pituitary failure.¹⁶

We believe that the hypothyroidism which these patients manifested was the result of primary inadequacy of the thyroid gland rather than "pituitary myxedema." The crucial test which could differentiate primary from secondary hypothyroidism, the response to TSH,¹⁷ was not performed. Whereas the clinical manifestations do not permit differentiation of primary from secondary hypothyroidism and the response to TSH was not studied, the presence of normal levels of TSH in the serum of Case 1 indicates that the pituitary was producing this hormone.

The reason for the primary pluriglandular failure which developed in these patients is not evident. Sclerosis of the endocrine glands (with the exception of the testes) was not found in Case II. It is possible that the patient reported as Case II had testicular failure because of aging (male climacteric) and then developed primary adrenal insufficiency (Addison's disease). The hypothyroidism then might have resulted from changes in the thyroid gland secondary to the absence of the adrenal hormones.¹⁸ However, the changes in the thyroid gland in this case were those of atrophy; fibrosis and lymphocytic infiltration which are characteristic of the changes in the thyroid gland in Schmidt syndrome¹⁹ were minimal. The morphologic changes in the endocrine glands in Case I are unknown, since autopsy was not performed. As already pointed out, the clinical findings and hormone assay values indicated primary adrenal, gonadal and thyroid failure.

The pluriglandular insufficiency in these two cases differs from the other reported cases of pluriglandular failure simulating hypopituitarism inasmuch as our patients did not have diabetes mellitus, and did have a greater degree of gonadal failure.

The male (Case II) had pulmonary fibrosis and heart disease in addition to hypothyroidism, hypoadrenalinism and hypogonadism. The cardio-respiratory disease was responsible for the difficulties experienced in the treatment of the adrenal failure with desoxycorticosterone. The changes in the electrolyte and water metabolism produced by small amounts of this drug were sufficient to precipitate congestive heart failure. Patients with adrenal failure complicated by heart disease are very sensitive to the electrolyte alterations produced by

desoxycorticosterone,²⁰ and may present difficult therapeutic problems. The chronic pulmonary disease producing chronic anoxia probably was responsible for the maintenance of a normal blood count in this patient in spite of the absence of hormonal factors which have erythropoietic-stimulating activity. Anemia may occur because of a deficiency of androgens²¹ or adrenal hormones²² or thyroid hormone,²³ as well as in panhypopituitarism.²⁴

SUMMARY

Two cases of pluriglandular insufficiency simulating panhypopituitarism are presented. Both patients had hypoadrenalinism, hypothyroidism and hypogonadism, with findings which indicated the presence of a functioning pituitary gland. In one of the two cases autopsy was performed and confirmed the presence of a normal pituitary gland.

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